A NOTE ON THE PRESENCE OF *OSTERTAGIA TRIFURCATA* IN THE ABOMASUM OF A SHEEP IN ENGLAND.

BY STORRAR CAVE, B.A.

(With 3 Text-figures.)

WHILE examining the strongyles of the fourth stomach of an ewe belonging to the Cambridge University Farm, which had been slaughtered for post-mortem examination, numerous male and female *Ostertagia circumcincta* (*Strongylus cervicornis* of MacFadyean) were found. A few specimens of *Haemonchus contortus* were also present.

Whilst mounting specimens of the male *Ostertagia circumcincta*, it was noticed that another strongyle was also present in the stomach, which resembled this species markedly, both in size and general form, especially in the bursa—but it differed in the spicules and gubernaculum, the former of these, instead of being long, narrow and bifid at their posterior extremities, as in *Ostertagia circumcincta*, are short, fairly broad and trifid posteriorly. The gubernaculum is narrow and lozenge-shaped.

After more careful examination there was no doubt that this other strongyle is identical with *Ostertagia trifurcata*—a species created by Ransom of the U.S.A. Bureau of Animal Industry in 1907, for a worm found in the fourth stomach of many sheep and goats in America. According to this author this species is always associated with *Ostertagia circumcincta*. No females of the worm were found by him, and I also have found none.

Specific Characteristics and Description.

The worm is 8·2 mm. long, gradually tapering in thickness from 18 μ at the head to a maximum of 110 μ just in front of the bursa. Two lateral cervical papillae 320 μ from the anterior end are present;

the excretory pore is just behind these, 310 μ from the mouth. The cuticle is transversely striated in the anterior portion of the body.

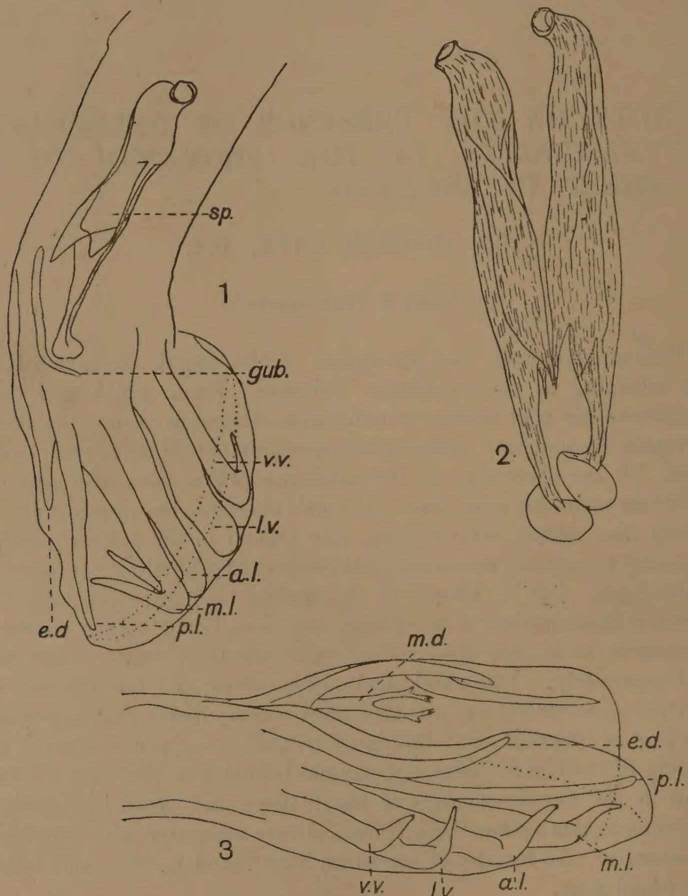

Fig 1. *Ostertagia trifurcata* (× 246 diameters). Lateral view of posterior end.
Fig. 2. *Ostertagia trifurcata* (× 432 diameters). Dorsal view of spicules.
Fig. 3. *Ostertagia trifurcata* (× 246 diameters). Dorso-lateral view of the bursa.

Reference figures: a.l.—antero-lateral rib; *e.d.*—externo-dorsal rib; *gub.*—gubernaculum; *l.v.*—latero-ventral rib; *m.l.*—medio-lateral rib; *p.l.*—postero-lateral rib; *sp.*—spicule; *v.v.*—ventro-ventral rib.

The bursa is very similar to that of the male *Ostertagia circumcincta*. It consists of two membranous wings 170 μ deep, supported on each side by externo-dorsal, postero-lateral, medio-lateral, antero-lateral, latero-ventral and ventro-ventral ribs ; of these the externo-dorsal and postero-lateral ribs are straight and narrow, the latter extending to the posterior limits of the bursa—while the remainder are curved and pointed at their extremities. The median dorsal rib, which is situated between the two externo-dorsal ribs, has the marked "antler" form as in *Ostertagia circumcincta*, but it differs slightly from the latter in that the lateral processes of the branches are situated rather more anteriorly. The stem of this rib is 50 μ in length, and each branch 40 μ.

The spicules are 180 μ in length (in *Ostertagia circumcincta* they are 300 μ) and the gubernaculum 100 μ.

The above dimensions are all somewhat greater than those given by Ransom ; the following list is a comparison :

Ostertagia trifurcata.

	Dimensions of Cambridge specimen	Dimensions given by Ransom
Spicule	180 μ	180 μ
Gubernaculum	100 μ	90 μ
Distance of cervical papillae from head ..	320 μ	300 μ
Distance of excretory pore from head ..	310 μ	290 μ
Total length	8·2 mm.	7 mm.
Width :		
head	18 μ	15 μ
maximum	110 μ	90 μ
Median dorsal rib :		
stem	50 μ	40–50 μ
branches	40 μ	35–40 μ

Unfortunately it was impossible to take these dimensions from fresh specimens, and these discrepancies may therefore be due to the swelling effects of the glycerin in which they were mounted—a possibility supported by the fact that the chitinous spicules, which are unlikely to be affected by this reagent, are identical in length. On the other hand these differences in size may be due to the different breed and environment of the hosts.

I have written this note since, as far as I am aware, the occurrence of *Ostertagia trifurcata* has hitherto only been recorded from America.

CALLIOBDELLA LOPHII.

By W. HAROLD LEIGH-SHARPE, B.Sc., A.C.P.

(With 5 Text-figures.)

Habitat. This marine leech, a member of the Rhyncobdellida, or jawless leeches, is parasitic on the angler, *Lophius piscatorius*, and, in my experience, attaches itself to the skin of the fish on the ventro-lateral surface indiscriminately; it is not found near the vent, as is usually the case with *Pontobdella*, which occurs frequently attached near the cloaca of the skate.

Record. *Calliobdella lophii* was taken by Hesse at Brest in 1863, by Lönnberg and Jägerskiold near Bergen in 1889, and by myself at Plymouth in 1913. It occurs in the Mediterranean (Blanchard), and on the coast of Norway (Johannson). In 1913 I published an account of my capture, and a historical survey, together with a description of the external characters only. Only such of the external characters as it is necessary to mention therefore will be repeated here.

Body. The leech is cylindrical, more or less spindle-shaped, broadest about the middle of the body. The animal is divided into two distinct regions, a neck region which is bare, and a body (in a restricted sense), the latter carrying laterally rounded vesicles described below. The body is elongated, slightly convex above, flattened below. Blanchard (1894) suggests it is flattened when young and rounded when old. The length is 5–7 cm. The colour is greenish-brown, with ventral rose-pink markings and an orange band, with other pigment characteristics, as described in my previous paper (1913).

Suckers. As is usual with leeches there are two suckers, one at either extremity of the animal. The anterior sucker surrounds the mouth, and the posterior sucker is characterised by its extreme size, being more than twice the maximum breadth of the body, and four

times the size of the mouth sucker. The circumstance that *C. lophii*, a rare leech, has been hitherto exclusively found on a single host, viz. *Lophius piscatorius*, leads one to the assumption that it is a completely stationary parasite which never leaves its host. With this are correlated the following facts: (i) in *C. lophii* the posterior sucker is much more powerfully developed than in *C. nodulifera*, which is known on a dozen hosts and has the posterior sucker only double the size of the anterior one, so that *C. lophii* is more adapted to hold fast to the body of the host; and (ii) the skin musculature is much more weakly developed in *C. lophii* than in *C. nodulifera*. Indeed the longitudinal muscle layer is so weakly developed in *C. lophii* that the animal can hardly swim freely. Both suckers are of a creamy-pink "flesh" tint, and are altogether paler and of a different complexion from the rest of the animal.

Segmentation. The neck consists of 22 annuli, but from the work of others I am forced to conclude that this number is not constant in the genus.

Blanchard describes *C. lubrica* as follows:

Neck: 3 small segments, followed by 11 large, more or less divided by chorisis, followed by 6 small.

The 2nd, 5th, 8th and last carry a small non-respiratory tubercle.

Clitellum: 2 large and 6 small segments; the male genital aperture being between the first and second, and the oviduct opening between the last but one and the last but two (?).

The *abdominal* somite is formed of 3 (?) or 6 rings, according as the 3 primordial rings are more or less divided by chorisis. *Twelve* (*sic*) respiratory vesicles, the first on the first double ring, the second on the fourth double ring, and so on. The anterior half of each double ring carrying the vesicles is marked on the dorsal face with whitish spots. After the last pair of vesicles one counts 8 rings of which the 4th and 7th carry white spots. The anus opens between the last and the last but one.

Compare with this the account given by Johannson (1896) of *C. lophii*:

"Each typical segment consists of 4 annuli, which are derived from one another by chorisis. The 1st and 2nd of these annuli which are broader than the others are often divided into two, so that there come to be 6. Often in this genus is every fourth ring distinguished by papillae."

There is no doubt that there are 6 annuli to an abdominal or body

segment, but whether they are derived in the manner stated by Blanchard or in that stated by Johannson I do not know, nor do I see on what grounds their statements rest, seeing that Johannson at least only examined preserved specimens. Comparing *Calliobdella* with *Pontobdella* it would seem that the first annulus of the segment in *Calliobdella* corresponds with the 1st and 2nd of *Pontobdella*, the 2nd and 3rd

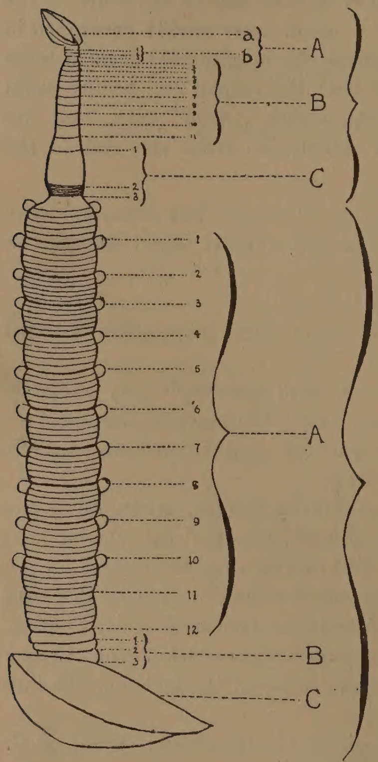

Fig. 1.

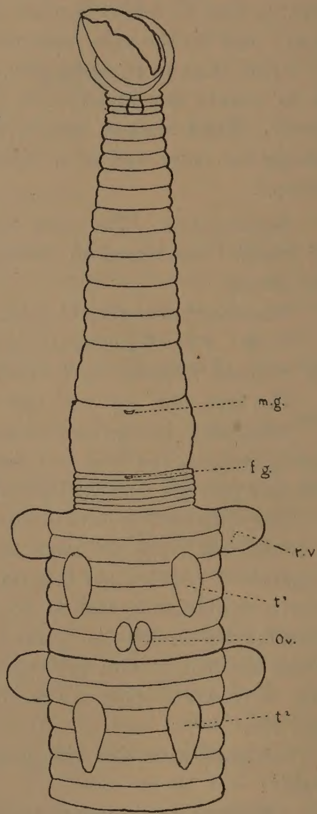

Fig. 2.

Fig. 1. *Calliobdella lophii.* The entire animal viewed from the ventral surface, to show the segmentations and annulations. Numbering and lettering explained in the text.

Fig. 2. The anterior portion of the same, viewed from the ventral surface, on an enlarged scale. *m.g.* male genital aperture, *f.g.* female genital aperture, t^1, t^2 the first two pairs of testes, *Ov.* ovaries, *r.v.* respiratory vesicles.

of *Calliobdella* with the 3rd and 4th of *Pontobdella*, and the 4th of *Calliobdella* with the 5th and 6th of *Pontobdella*.

Judging from my specimens, the diagnosis is as follows, which is made plain on reference to Fig. 1 :

(α) *Neck.* A. HEAD : 5 or 6 segments.

(*a*) *Anterior Sucker :* which shows annulations which gradually fade away and grow more indistinct anteriorly.

(*b*) 3 *small annuli* each of which is a complete segment.

Total 9 (or 8) segments.

B. PRECLITELLUM :

11 *annuli*, all more or less divided by chorisis (except the first 3 or 4), in many cases the division not extending right across the annulus, so that the number of subdivisions of the annulus does not always appear to be the same on both sides of the specimen. The whole of the 11 annuli only represents 3 segments. The preclitellum is divided from the clitellum by a deep furrow which shows as a very marked line in a preparation cleared in creosote, viewed under the microscope as a transparent object.

C. CLITELLUM : 3 segments.

The first two segments are very large and not divided into annuli by furrows, though the edges of the animal, viewed as a flat object, show indications of subdivisions. The male genital aperture is between these two segments or somewhat towards the anterior border of the second. It is this segment that bears the orange band. The third segment is small, and is divided into six minute annuli which are not again divided.

The female genital aperture is between the second and third segments.

I do *not* find any lateral tubercles on the neck whether respiratory or otherwise.

(β) *Body or Abdomen.* (A) TESTIS REGION AND CAECUM REGION TAKEN TOGETHER. 12 segments.

Each of the 12 segments is completely divided into 6 annuli. The first 11 only, of these 12, bear lateral respiratory vesiclés one on either side of each segment, bordering the first two annuli of these

segments. The 12th segment though not bearing vesicles is also divided into 6 annuli.

(B) Anal Region:

3 annuli, each of which is a complete segment, the anus opening between the last and the last but one.

(C) Posterior Sucker: which probably represents 7 segments.
Total for the whole body 34 segments.

Nervous System. There is, of course, a ventral nerve cord with ganglia along the chain. The foregoing conclusions concerning segmentation (not annulation) are based principally on the consideration of these ganglia. Whitman (1885) was the first who gave an account in which he distinguished the number of segments in leeches. In the common leech (*Hirudo*) he made this number 23. Bourne (1884) also regarded the ganglia of the neck mass and of the posterior sucker respectively as one complete ganglion, making a total of 23 ganglia. But these are undoubtedly large ganglion masses formed by the fusion of several ganglia. Leuckart (1894) considers that the anterior ganglion is equivalent to 5 ganglia, while the posterior ganglion is equivalent to 7 ganglia.

But Whitman (1889) now shows us that the upper part of the anterior ganglion (oesophageal ganglion) indicates a separate segment.

$$23 - 2 + 5 + 7 + 1 = 34 \text{ ganglia.}$$

Respiration. Respiration is carried on by means of rounded vesicles protruding from the abdominal portion of the body. Considering that Van Beneden and Hesse (1863) mention two different numbers of pairs of vesicles and figure yet a third number, it is no wonder that there is some confusion on the subject. Blanchard states that there are 12 pairs, and so there may be in other specimens. But I am quite prepared to agree with Johannson that there are *eleven* pairs in *Calliobdella*, and most certainly so in *C. lophii*, which is not one of the numbers given by Van Beneden and Hesse. These vesicles rise and fall. They are rudimentary branchiae corresponding to the large external branchiae of *Branchellion*, and similar to the hemispherical vesicles of *Cystibranchus*. According to Quatrefages (1852) these appendages do not receive the blood contained in the vessels but only the lymph, which becomes diffused so that the respiration is truly lymphatic.

Pigment. The blackish-brown star-shaped pigment cells which are characteristic of *Piscicola* and *Cystibranchus* are absent in this

genus. According to Johannson the clear yellow pigment cells which are present in *C. nodulifera* (the only other species which he has examined) are entirely absent. They are, however, replaced by similar cells with brownish contents, for in section can be seen, in the inner connective tissues at least, large cells which in position and form are somewhat like the pigment cells of *C. nodulifera* but have a very pale grey-brown colour.

Digestive system. The mouth is situated at or near the centre of the anterior hood-like sucker, and the anus opens on the dorsal side between the last and last but one of the annuli immediately preceding the posterior sucker. The oesophageal bulb or pharynx is very thick, its opening being furnished with denticulations or folds destined to favour contraction. The mouth can be shut so as to form a longitudinal slit, and its wall is the anterior sucker. The pharyngeal sheath opens with a fine opening at the bottom of the mouth cavity, and consists of a narrow tube whose lumen is either wholly or partly filled by the pharynx, and when the pharynx is retracted it stretches to the end of the first clitellar segment. Without the mouth cavity the pharyngeal sheath for the whole of its length is in the body cavity. Salivary glands are present. The stomach is the longest part of the alimentary canal; it is wide in the testis region and divided into chambers, the front part of each segment being constricted by dorsiventrally placed muscular septa which occur in all. These chambers increase in width backwards. As is usual in leeches a large caecum is present, which has in *C. lophii* always been found very empty. Very wide in other species, in this species on the contrary it is strongly flattened. Apparently it represents two caeca fused, the point of division of two original caeca being represented merely by two fine tubes of which one is often obliterated. The whole caecum exhibits thus a shrunken aspect. The part I have called the intestine (Fig. 4) is the only tract in my specimens that shows any trace of contents. This is another argument in addition to the two points I have mentioned under the heading of "Suckers" in favour of the view that *C. lophii* is a stationary parasite, as it does not seem to gorge itself with food, which would be necessary for a leech which detaches itself, during the period of time while it was searching for a new host.

Coelôm. The body cavity is divided into the five following parts: a ventral sinus, a dorsal sinus, two lateral sinuses, and an intestinal sinus.

The *ventral sinus* is the best developed. It commences at about

the border of the mouth part and the oesophageal part of the alimentary canal and includes the pharyngeal sheath down to its conclusion. After the pharyngeal sheath leaves the oesophagus it runs, not as in other genera freely inside the ventral sinus, but in front of the oesophageal ring, and follows along with a connective tissue sheath which surrounds the pharyngeal sheath to its conclusion, separated

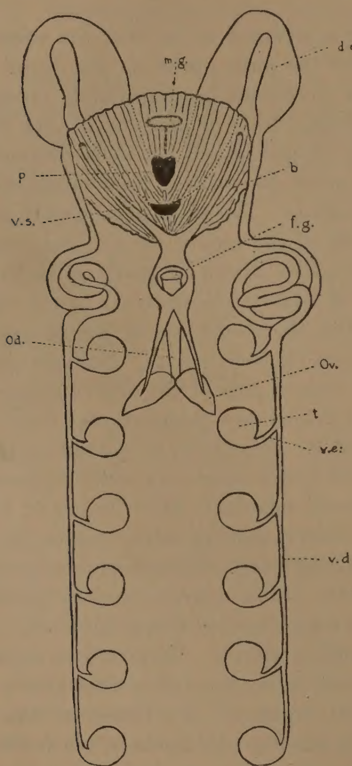

Fig. 3.

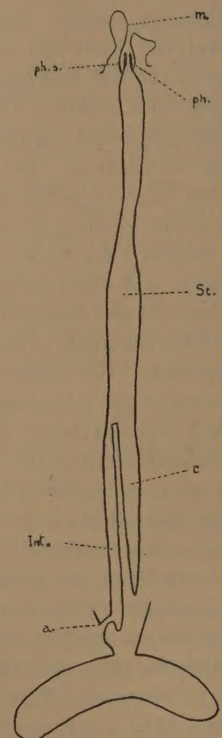

Fig. 4.

Fig. 3. The reproductive system of *Calliobdella lophii* viewed from the ventral surface. *t.* testes, *Ov.* ovaries, *v.e.* vasa efferentia, *v.d.* vasa deferentia, *d.e.* ductus ejaculatorii, *b.* bursa, *p.* penis, *Od.* oviduct, *m.g.* male genital aperture, *f.g.* female genital aperture, *v.s.* vesiculae seminales.

(For the sake of the diagram the testes are too close together, below *f.g.* the proportion is not kept. For relative scale see Fig. 2.)

Fig. 4. Diagrammatic lateral view of the alimentary canal, *ph.* pharynx, *ph.s.* pharyngeal sheath, *St.* stomach, *c.* caecum, *Int.* Intestine, *m.* mouth, *a.* anus.

from it by a cavity which stands in communication with the ventral sinus. It stretches to the posterior sucker situated on the ventral side of the stomach and caecum. It includes the ventral ganglion chain in all its length. The hinder ganglia are surrounded by a separate part of the body cavity separated from the ventral sinus by a septum. In this genus it includes the ventral blood vessel. In the clitellum it is narrow, but in open communication with a cavity which includes the male and female ducts, and which is constricted between the ganglia.

Reproductive System. The generative organs are situated at the base of the neck in the middle of which the opening of the oviduct is situated between the 2nd and 3rd clitellar segments or towards the anterior border of the 3rd, while the male genital aperture is between the 1st and 2nd clitellar segments, or towards the anterior border of the 2nd.

The *male* reproductive organs consist of metameric testes and efferent ducts, situated in the first six segments of the abdomen. Van Beneden and Hesse were uncertain in another species as to whether there were seven or eight pairs of testes, but apparently they counted the ovaries in their reckoning, for in common with all the Ichthyobdellida (with the exception of *Abranchus*) there are but *six* pairs of testes.

From each testis, as is usual, proceeds a small duct, the vas efferens; there being as many vasa efferentia as there are testes. From the posterior testis on each side the vas efferens passes immediately into the vas deferens which in its course receives the remaining vasa efferentia. The vasa deferentia are in the testis region very fine tubes little or no larger than the vasa efferentia, but have a larger lumen. Only after taking up the most anterior vas efferens each vas deferens becomes a little larger. In the posterior part of the clitellum it takes on many curves and sinuosities, as it does also slightly to some extent in *Piscicola*, while in most genera it is perfectly straight. It gradually gets a covering of more and more powerful circular muscles. The borders of the epithelial cells are always very obscure, while the nuclei are very conspicuous. About the male genital aperture the vasa deferentia suddenly widen out and open into the ductus ejaculatorii.

The ductus ejaculatorii stretch forward to the anterior border of the clitellum, after which they bend towards the ventral side and posteriorly in order to unite with one another in the middle line in front of the female genital opening. The common terminal portion turns a considerable way backwards and opens only a little anterior to the female opening into the bursa; in the other genera this common terminal portion does not stretch so far backwards but opens very

quickly into the bursa. In the forward-going part of each ductus ejaculatorius the epithelial cells are large and in general very indistinct, and their nuclei observable also only with difficulty. The epithelium is surrounded by a very powerful circular muscle layer. The cells here commence by degrees to become secreting cells, and in the backwardly directed part of the ductus ejaculatorius they become more and more obviously such, and at the same time more indistinct.

The bursa is a very characteristic feature of *Calliobdella*. This is a saccular organ into which the common end part of the ductus ejaculatorii opens, and which opens outwards by means of the male genital aperture on the second clitellar segment, through which it is able to be everted, and is distinguishable from that of other genera by its enormous and peculiar development. It is a flattened sac whose wall is considerably plaited, an especially large fold going forward immediately in front of the openings of the ductus ejaculatorii. The inside of the bursa is lined with epithelium consisting of high narrow cells. The musculature is extremely powerful; partly because the wall of the bursa is covered with large muscle threads crossing each other, which form duplicated layers, partly because there go out from the muscle layer both to the dorsal and ventral sides, giant muscle fibres often having a diameter of 40–50 μ, and containing nuclei up to 25 μ in diameter, which size is important in comparison with other leeches. These muscles send out branches with which they root themselves fast both among the muscles of the wall of the bursa as well as the walls of the body. The wall of the bursa towards the dorsal side immediately behind the opening of the ductus ejaculatorius is developed in a peculiar manner so as to form a spherical organ ·3 mm. in thickness, which is a process of the cavity of the bursa flattened and conical in form directed dorsally and forwards. The bursal epithelium is here small celled, under it is a thin layer of loose connective tissue, afterwards a very firm connective tissue membrane, in which are fastened muscles which stretch towards the periphery of the organ, where they fasten themselves to a similar membrane. Outside around this again lies a layer of loose connective tissue which contains fine muscle threads. For most of these histological details I am indebted to Johannson.

The whole bursa, except a little region in the neighbourhood of its opening, is surrounded by a portion of the body cavity which even extends forwards round the ductus ejaculatorii. With the help of the above-mentioned muscles the whole bursa can be everted and forms a penis capable of standing out at right angles to the body, which plainly must

perform the function of a copulatory organ. This is heart-shaped and includes the whole of the ductus ejaculatorii, which open on a point immediately on the summit of the above-described muscular organ, which directs its aperture towards the opening of the ductus. Even the whole glandular mass is included in the penis, and it is chiefly this which gives it its form. The penis transports spermatophores under copulation, being well adapted for such a function.

Into the antero-lateral divisions of the bursa there open two small tubes which extend out over the ventral side of the bursa, and gradually approaching each other they fuse together immediately in front of the female genital aperture. After they fuse they separate once more, but unite yet again a little behind the oviduct, after which they quickly separate again and run separately until they terminate. They are narrow in the anterior portion, but much wider at the point of connection. They lie close to the ovaries on their ventral side, and extend over the same distance as these to the first and second abdominal segments. These tubes Johannson (1896) and Olsson (1876) found full of spermatozoa and are to be regarded as vesiculae seminales. Their openings into the bursa are so situated that when the bursa is protruded they lie one on each side. The possession of vesiculae seminales is peculiar to the genus *Calliobdella*.

The *female* reproductive organs consist of a pair of ovaries which extend usually to the border of the second, and sometimes to the third abdominal segment. They unite in front in the middle line and open out into an oviduct, which in this genus goes through the ring which is formed by the vesiculae seminales coming together, both immediately in front and immediately behind the oviduct.

Eggs. According to Hesse, all the species of *Calliobdella* that he kept in captivity laid eggs freely. Those of *C. lophii* were oval, about 1 mm. in length at their greatest diameter, and adhered to the walls of the vessel in which the leeches were kept by a gelatinous secretion; they much resembled the cocoon of *Bombyx mori* and were of a yellow colour, and their surface was covered with very crisp and curly "silk." The eggs of all the other species are, according to the same observer, hemispherical.

General Note. The disagreement between the descriptions of Van Beneden and Hesse and his specimens led Blanchard to place them in the genus *Trachelobdella*, but the great differences between Blanchard's diagnosis and that of Johannson can only lead me to the conclusion that they have been examining quite different animals. For instance,

inter alia, Blanchard found non-respiratory vesicles on the preclitellum, and 12 respiratory vesicles instead of 11. I have no doubt that Johannson and I have been describing the same animal, which is the *Calliobdella lophii* of Van Beneden and Hesse. The confusion originally arose out of Van Beneden and Hesse's faulty counting of the respiratory vesicles, and giving in their figures a different number from that mentioned in their text.

I allude to this more particularly since W. A. Harding (in *Parasitology*, Vol. III. No. 2, 1910) in his paper on the Hirudinea, describes an animal which he calls *Trachelobdella*, and in his descriptions follows

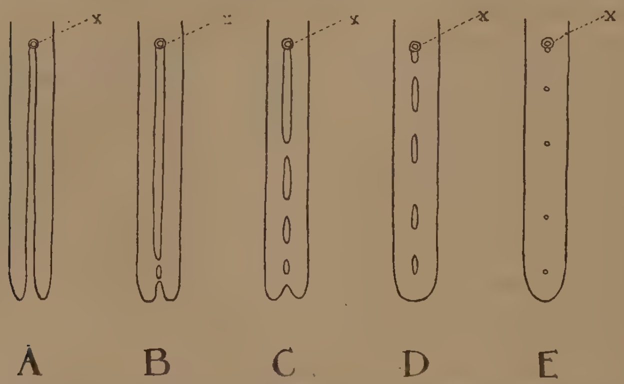

Fig. 5 (after Johannson). A comparative scheme of the condition of the caeca in various Ichthyobdellid leeches, showing the reduction from two to one.

A. *Abranchus brunneus*. *B*. *Abranchus microstomus*. *C*. *Platybdella anarrhichae*. *D*. *Piscicola geometra*. *E*. *Calliobdella nodulifera*.

X. The aperture from the stomach.

Blanchard, upholding that author rather to the disparagement of Johannson. Harding mentions catches, one by T. Scott in Scotland in 1901, which probably were *Trachelobdella*, though the number of respiratory vesicles is uncertain, and the reproductive system does not appear to have been investigated. Harding follows Blanchard in good faith, but I cannot uphold him in saying that the genera *Calliobdella* and *Trachelobdella* are one.

Another alternative remains, namely that the number of respiratory vesicles is variable; but then one would have to suppose that the presence or absence of non-respiratory vesicles on the preclitellum is variable also. I think it is more than a coincidence that makes the

12-vesicled animal have the non-respiratory vesicles, and the 11-vesicled animal be destitute of them.

Confusion also arises in the descriptions, because writers on leeches will not be careful enough to differentiate between segments and annuli, often using the word " rings " to cover both, leaving it to the imagination of the reader to discriminate which is meant.

Summary of the characters leading to the belief that Calliobdella lophii *is a perfectly stationary parasite never quitting its host.*

1. Extreme size of the posterior sucker.
2. Weak development of the longitudinal muscle layer.
3. Its occurrence on only one host.
4. Several members (5 and 4) being found together on one host, hence rendering fertilisation possible.
5. The extreme care with which the living leech, when moving, places the posterior sucker as exactly as possible in the position previously occupied by the anterior (as observed by Hesse and myself).
6. Reduction from two caeca to one.
7. Shrunken aspect of caecum, with lumen partly obliterated.
8. Empty condition of caecum, and alimentary tract in general.
9. Its rarity.

Conversely the stationary habit of the leech satisfactorily accounts for its rarity.

These suggestions are of importance, inasmuch as though we have a new parasite on our coasts, it is not likely to spread.

Generic characters. A leech divided in a marked manner into a neck and a body. Each body segment consists of 6 annuli. Along the side of the body are *eleven* pairs of pulsating respiratory vesicles. The common terminal portion of the ductus ejaculatorii opens into a large and wide bursa which can be protruded, wherewith is formed a copulatory apparatus which is at least as long as the breadth of the body in the same place, and has at its end a peculiar muscular part. A pair of vesiculae seminales are present. There are no eyes. Blackish-brown star-shaped pigment cells are absent.

Specific characters. Van Beneden and Hesse separated 3 species : *C. lophii*, *C. punctata*, and *C. striata*. Other authors recognise 4 or only 2, giving them various names, but *all* retain the species *C. lophii*. Apparently its only host is *Lophius piscatorius*. It is characterised by the extreme size of the posterior sucker, it being more than twice

the maximum breadth of the body, *and four times* the size of the mouth sucker. The body is not bestrewn with yellow dots. Immediately behind the neck the body increases in breadth more rapidly than in *C. nodulifera*, so that the relative difference between the two parts is more marked, and the flattened condition of the abdominal part is thus the more strongly noticeable, a condition which is probably dependent on the weak development of the musculature of that part. Eggs oval.

The unmistakable colouration characters may be of specific value.

(This investigation was carried out at the South-Western Polytechnic Institute, Chelsea, London, S.W. I have to acknowledge the kind help of Mr J. T. Cunningham in checking the results; also of Mr Harold R. Southam for reproducing the figures from my rough sketches.)

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LEIGH-SHARPE (1913). *Calliobdella lophii. Journ. Mar. Biol. Assoc.*, X. No. 1. p. 81.

LEUCKART (1894). *Parasiten des Menschen*, I.

OLSSON (1876). Bidrag till Skandinaviens Helminthfauna. *Vet. Akad. Handl.*, I. and *ibid.* II. (1893).

QUATREFAGES (1852). *Ann. Sc. Nat.* XVIII. p. 322.

VAN BENEDEN and HESSE (1863). *Recherches sur les Bdellodes ou Hirudinées.*

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— (1889). Some new facts about the Hirudinea. *Journal of Morphology* (2),

APPENDIX.

An attempt to separate the genera Calliobdella *and* Trachelobdella.

I. *Records and Literature.*

CALLIOBDELLA

Brest.—VAN BENEDEN and HESSE (1863), *Recherches sur les Bdellodes ou Hirudinées.*

Coasts of Norway (Bergen, Helsö etc.).—Genus amended and defined by JOHANNSON (1896), *Bidrag till kännedomen om sveriges Ichthyobdellider,* Upsala; also JOHANNSON (1896), *Die Ichthyobdelliden in Zool. Reichsmuseum in Stockholm.*

Respiratory vesicles defined as 11 pairs in number, 6 annuli to abdominal somites and no vesicles on the preclitellum.

Plymouth.—LEIGH-SHARPE (1913), *Journ. Marine Biological Association,* vol. X. No. 1.

Work of Johannson confirmed.

Seas of Japan (?).—Two new species described by OKA, *Annot. Zool. Jap.* (1910), vol. VII. p. 174, but as he defines these as having 5 annuli to the abdominal somite, and 13 pairs of respiratory vesicles they may be at once ruled out, though described as *Calliobdella.*

TRACHELOBDELLA.

(?) *Brazil.*—DIESING (1850).—*Systema Helminthum,* I.

Mediterranean.—BLANCHARD (1894), "Hirudinées de l'Italie," *Boll. Mus. Zool. Torino,* vol. IX. No. 192.

Genus defined with 4 non-respiratory vesicles on the preclitellum, respiratory vesicles 12 pairs in number, 3 or 6 annuli on the abdominal somite, described as *Calliobdella,* but afterwards the author merges *Calliobdella* with *Trachelobdella,* **defining respiratory vesicles 12 to 14 pairs.**

Scotland (?).—McINTOSH (1874), *Invertebrates of St Andrews.*

—— (?).—SCOTT (1901), "Parasites of Fishes," 19*th Annual Report Fish. Board of Scotland,* vol. III. p. 138. Both these reports give the respiratory vesicles as 12 to 14 pairs, rest of description vague, may possibly be *Trachelobdella.*

Seas of Japan (?).—New species described by OKA (1910), *Annot. Zool. Jap.* VII. p. 173, defined with 6 annuli to the abdominal somite, and 13 pairs of respiratory vesicles, may possibly be *Trachelobdella.*

Turkestan.—ŠČEGOLEV (1912), *Trd. gidrobiol. St. Glubokoe, Moskva,* vol. IV. (I have not been able to obtain access to this Paper.)

II. *Summary of the principal differences between Blanchard's description and mine.*

(1) Respiratory vesicles 11 pairs.	(1) Respiratory vesicles 12 pairs (afterwards altered to 11 or 12 pairs, after examining specimens diagnosed by others as *Trachelobdella*).
(2) No non-respiratory vesicles on the preclitellum.	(2) Four pairs of non-respiratory vesicles on the preclitellum.
(3) Preclitellum of 11 annuli.	(3) Preclitellum of 9 annuli (not so stated).

(4) Clitellum of 3 segments, the first two large, and the 3rd divided into 6 small annuli.	(4) Clitellum of 3 segments, the first two large and the third divided into 6 small annuli (not so stated and mistranslated by Harding).
(5) Male genital aperture on first large segment of the clitellum.	(5) Male genital aperture between the 1st and 2nd annulus of the 3rd segment of the clitellum.
(6) Female genital aperture on the first annulus of the 3rd segment of the clitellum.	(6) Female genital aperture between the 4th and 5th annulus of the 3rd segment of the clitellum.
(7) Vesiculae seminales present.	(7) (Unknown.)
(8) Caeca fused to form one caecum.	(8) (Unknown.)
(9) Colour (orange band, rose-pink V's etc.), agreeing with the description of Van Beneden and Hesse.	(9) (Unknown.)
(10) Abdominal somite of 6 annuli (4–6—Johannson).	(10) Abdominal somite of 3–6 annuli.
(11) *If Calliobdella* had 12 respiratory vesicles (which it has *not*) after the last one would count but 7 small rings; four more to complete the somite, and three others (see Fig. 1).	(11) "After the last respiratory vesicle one counts 8 small rings."
(12) No trace of any preputial arrangement, not even in post-mortem contraction.	(12) The base of the neck inserted into the body and surrounded by the latter so as to form a prepuce.

ON A SECOND AND THIRD CASE OF INFECTION WITH *PLEROCERCOIDES PROLIFER* IJIMA, FOUND IN JAPAN.

BY S. O. YOSHIDA,

Pathological Department of Osaka Medical Academy.

(With Plate XVII.)

THIS peculiar larval form of cestode, parasitic in man, was first discovered in a Japanese woman (Case I) on 9. vii. 1904, and described by Prof. Ijima in 1905. Subsequently it was found in an American by Dr H. Gates, of Manatee, Florida, in June, 1907, and described by Dr Charles Stiles, who had received Gates' specimens for identification on 25. v. 1908. In the same year in which Gates' case was recorded, we observed a second case (Case II) in Japan. In this case an enormous number of worms were present and they were generally larger than those from the former Japanese and American cases. Recently a patient suffering from the same form of cestode larva (Case III) was found in the Dermatological Institute of the Imperial University of Kyoto.

The first and third Japanese cases are similar to one another in respect to the characters of worms, whereas there is slight difference in the symptomatology. But the second case is very different from both the foregoing cases. I shall here chiefly describe the second Japanese case, which I have personally observed and examined, and shall add a brief account of the third case, which I have not studied personally, but regarding which I have received information from the physician who attended the patient.

I am much indebted to Prof. Ijima, and to Dr I. Aoyama, Professor of the Medical College, Imperial University of Tokyo, as well as to his assistant, Mr Usui, for the help they have given me. The former has

superintended my work and allowed me to use his numerous specimens prepared from Case I. The opportunity of studying the parasite and all information about the patient in Case II were given by the latter two gentlemen. My thanks are also due to Mr Inoue who gave me valuable information about the worms and the patient in Case III.

The second case in Japan.

An enormous number of the cestode larvae were obtained from a patient who died of a parasitic disease in the Imperial University of Tokyo Hospital, on 13. xii. 1907.

The patient and his symptoms. The patient, a monk, 36 years old, Bunkai Shibasaki by name, had settled recently in a temple in Tokyo. I am unable to give a detailed account of his life before his coming to the hospital. His statements show that some six years previously he had suffered from a disease the cause of which neither himself nor his family understood. This was the origin of his fatal illness. About a year previous to his coming to the hospital, he accidentally scraped a worm out of his neck region. In the year 1907 the disease became serious and produced in him an unusual state of mind, probably because his brain was attacked by the worm.

He came to the University Hospital 13. xi. 1907. He appeared thin and anaemic. One glance at him showed the presence of the worms under the skin as indicated by the presence of vermicular or knob-like swellings of the integument. On close examination these cutaneous swellings were found in every part of the body, *i.e.* upon the face, trunk, and both upper and lower extremities. When I visited the patient on 11. xii. 1907, I found the swellings more plentifully on the breast and the abdomen than in any other parts. According to the information given by Mr Usui, who attended the patient and observed him carefully, the number of cutaneous swellings in the various parts of the body varied periodically. This observation shows that the worms were moving about in the underlying tissues. There was nothing remarkable about the symptoms from their pathological aspect; they differed entirely, however, from those observed in Case I. In Case I, the patient's skin itched at the spots under which the worm capsules were present, as mentioned by Prof. Ijima, but in Case II the patient did not complain of itching; it should be noted, however, that the patient's sensibility may have been lowered because of the worms attacking the brain. I shall not, however, dwell on the symptoms since they lie outside my province.

Abundance of the worms. At autopsy, a considerable number of the parasites were found not only in the subcutaneous fat, and muscles, but in the walls of the visceral organs including the alimentary canal, mesentery, kidney, lung, and heart, and a few were even encountered in the ventricle of the brain. The worms were not present in the spinal cord, the cavities of the heart, and in the orbit. The parasites were uniformly so abundant that we could not cut any portion of the tissues without injuring some of the worms. Fig. 3 represents a piece of muscle taken from the patient's back and showing many worm capsules on its surface. The number of the parasites in the whole body is of course innumerable. In my specimen of muscle from the lumbar region, I can easily count 20–25 capsules in every 3 cm. square. We found rather fewer worms in the subcutaneous tissues of the head. It will be easily understood that death was caused by the great abundance of the parasites. Most of them were in the encapsuled state, but some were free in the tissues.

Capsules. The worm capsules were very widely distributed in the body, as mentioned above. In Case I, only the cutaneous tissues were examined, the muscular tissues and viscera were left untouched. It is reasonable to believe, however, that if the worm can divide and increase in number in the body, they will have infested the internal organs and ultimately have caused the death of the patient. We cannot, however, supply any further information regarding the subsequent history of Case I, because the patient left the University Hospital. In Case I, the infested tissues—subcutaneous, chorion, and connective tissue—were greatly thickened, while in Case II there was no remarkable change in the infested tissues.

The size of the capsules varies greatly according to the nature of the tissue, in which they occur; some are small and globular and measure about 1 mm. in diameter, whilst the largest measure 16×5 mm.; the capsules commonly measure 3–6 mm. in length and breadth. The large and the small capsules do not occur in any special localities; they were found mixed together in all portions of tissues. The capsules are spherical, ovoid or elongated in shape, the ovoid form being the most common. Figs. 1 (n, m, o, p, and q) and 2 (A) represent various sizes and shapes of capsules. Each capsule is either isolated in the tissues or connected with others, and usually contains only one parasite, but sometimes two or more are found in it. In the fresh state it was easy to remove the capsules from the surrounding tissues. The wall of the capsules was yellowish-white in

colour and tolerably thick. If an opening was made in the capsular wall and the capsule gently pressed with a finger or handle of a knife, the worm could be pushed out unharmed together with the surrounding fluid. An enormous number of the parasites were pressed out during the surgical operations, but they were nearly all injured. Fig. 2 shows the numerous capsules in the muscular tissue and the worm hanging out from the capsule. The specimens were obtained by pulling them out with the utmost care from the uninjured capsules, making small openings on the walls, and then squeezing out the worms.

Specimens of worms and capsules. The specimens of worms which were fixed in cold sublimate (at room temperature) or alcohol, underwent considerable shrinkage. At the suggestion of Prof. Ijima, I put some worms freshly removed from capsules, into slightly warmed physiological salt solution when they slowly moved and extended their bodies to the full length. These fully extended worms were then quickly transferred to hot sublimate; and thus good specimens were obtained. Upon removing the capsules from the tissues they were fixed in cold sublimate or alcohol; large pieces of tissue—muscle and fat—were fixed in 5 % formalin, and the small piece in Müller's solution.

Size and shape of the worm. Various sizes and shapes of the worms are represented in figs. 1 and 2. It is noticeable that my specimens are very large in comparison with those from Case I. The largest specimen measures 75 × 2 mm. in length, a second 33 × 2 mm., both being in a somewhat contracted state; a third specimen measures 25 × 2 mm. in the contracted condition and a fourth specimen only measures 2 × 4 mm. The most remarkable point in the shape is the budding or branching. The division of the worm within the capsules, and the process of budding out and forming a supernumerary head, were described in detail by Prof. Ijima in the preceding case. But among my numerous specimens, there are various forms of budding which we could not find in Case I (fig. 2, *h*, *f*). The specimens represented in fig. 2, *f*, and *h*, possess well developed buds or branches, and it is difficult to distinguish the original head from the supernumerary. The budding seems to take place in any part of the body, as represented in figs. 1 and 2. The size of the worm possessing the buds is also indefinite and we may find the buds on both small and large individuals.

Extremities. The sucker-like or groove-like depression of the frontal end in many specimens is very conspicuous, especially in

strongly contracted specimens (fig. 1, *J*). It is not a permanent sucker or groove comparable with those in other cestodes, but only an anterior invagination temporarily formed by the contraction of the head region. It is probable that this anterior invagination functions as a sucker. The contracted form of the posterior end is always different from that of the anterior end and the worm is unable to attach itself by this extremity.

The internal structure. So far as my investigation goes, the internal structure of the parasite is essentially similar to that of the worms from Case I. I may, therefore, limit myself to the following remarks:

The cuticula is very thin, generally not exceeding about 0·005 mm. in thickness. The fine dermal musculature lying under the cuticula consists of an outer layer of circular and an inner layer of longitudinal fibres. The nutritive particles, so termed by Prof. Ijima, vary in size and number; their shape is spherical, ovoid, or cylindrical. At first only a few of them were found in each specimen and this caused me to think for a time that the parasites possess fewer of them than do most cestodes. Subsequently, however, I found many specimens possessing an enormous number of them. It seems to me that the size, number, or position of the nutritive particles is irregular, but I have not yet arrived at any conclusion concerning the factors which determine these points.

The calcareous bodies are not so abundant; they are spherical or ovoid in shape, measuring 0·01 mm. in length by 0·005–0·007 mm. in breadth. They are equally distributed in all parts of the body, with the exception of the young buds in which only a few of them were found.

The parenchymal musculature, the excretory canal, and other structures are the same as those described by Ijima in Case I.

We tried to transplant the worm into the subcutaneous tissues of a monkey for the purpose of determining if it is capable of proliferation in the tissues of other mammals. The experiment was tried by Dr Aoyama's assistant, but the monkey died soon afterwards from some unknown cause, the result being negative.

The third case in Japan.

In 1911 a third case of infection with this cestode larva was observed in the Dermatological Institute, Imperial University of Kyoto. Unfortunately, I did not see the case, but by the kindness of Mr Inoue, I have examined worms removed from the patient and

assumed that they are identical with the proliferating cestode larvae found in Cases I and II.

The worms in Case III are almost all small in size, and possess few buds or branchings. The size and shape of the worms is nearly the same as in Case I, but is very different from those in Case II. Hence there is no need to mention the external features and internal structures of the worm in Case III.

History of Case III (supplied by Mr Inoue). The patient is a man 57 years old, Kyukichi Ohyabu by name, living in Kyoto. About 13 years previous to his coming to the hospital, he discovered accidentally a few nodules in the skin of the inner side of the right thigh. Since then the number of nodules gradually increased and they were found in many other parts of the body. The nodules varied in size. In the earlier course of the disease, he felt itching of the skin at the seat of the nodules, and he sometimes noticed a white vermicular mass scraped out by his own scratching.

He entered the University Hospital on 30. v. 1911, to obtain treatment for a remarkable swelling of the right lower limb. The patient was well nourished and in a healthy condition except for the presence of a hernia inguinalis on the right side. In all parts of the body, with the exception of the head and face, we found the numerous nodules of varying sizes, some of them standing out from the surface of skin, and others imbedded in the subcutaneous tissues. The former were indicated by the swelling of the integument, and by the thinning out and pigmentation of the cortical layer of skin; the latter were of hard texture and easily recognized by touching the skin. It was easily observed, as in the preceding cases, that the vermicular nodules slowly changed their positions, according to the movement of the worms through the subcutaneous tissues.

The swollen portion of the right thigh gave the appearance of elephantiasis, and the swelling was evidently caused by an accumulation of pus in the subcutaneous tissues. On removing the pus, the swelling gradually diminished and ultimately disappeared. He left Hospital 21. vii. 1911 when the swelling was cured.

On 30. v. 1913, however, he returned to the Hospital on account of recurrence of a more serious swelling of the right thigh. He now appeared well nourished and seemingly healthy, except for this swelling. Vermicular nodules, cutaneous and subcutaneous, were, however, found in all parts of the body and were considerably more numerous than before. As formerly, the swelling was easily cured by

Fig 5

Fig 1

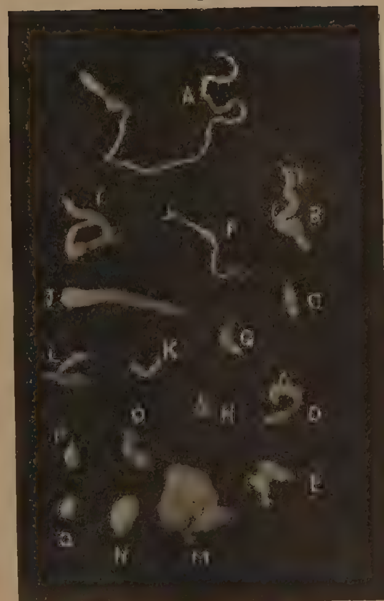


Fig. 3

Fig 4

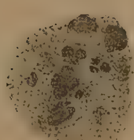
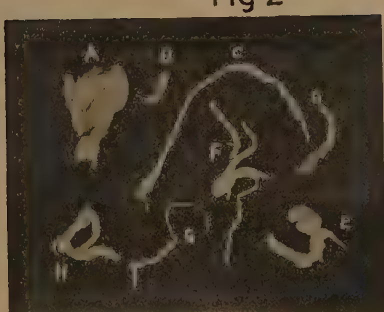


Fig 2



pressing out the pus accumulated in the underlying tissues. This time a few worms were found in the pus. As soon as the swelling disappeared, he left the Hospital (23. vi. 1913) without undergoing any treatment for the worms.

The patient is still alive (June 1914).

EXPLANATION OF PLATE XVII.

Figs. 1 and 2. Worms and capsules. Showing various shapes and sizes. Nat. size.

Fig. 3. A piece of muscle from the dorsal region. Nat. size. *C* Capsules, *W* Worm

Fig. 4. Nutritive particles. ×400.

Fig. 5. Section of capsules. ×300. *M* muscles. *NM* nutritive matter. *W* wall of capsule.

INGESTION OF ERYTHROCYTES BY TRYPANOSOMES.

By HARALD SEIDELIN, M.D.

(With Plate XVIII.)

On examining fresh specimens of white rats' blood containing numerous trypanosomes of a strain (*T. brucei* group), for which I am indebted to Dr J. W. Scott Macfie and with which I was experimenting at the Medical Research Institute at Yaba, Nigeria, I was struck by the energetic way in which the parasites attacked the red blood corpuscles. They sometimes adhered to the corpuscles for a long time, moving them about in different ways, and sometimes appeared to pierce their membrane, now in one place, now in another, penetrating more or less deeply into their substance. A careful search of a number of stained films revealed in some the presence of very interesting appearances which seem to establish the important fact that these trypanosomes are capable of ingesting and digesting erythrocytes. The accompanying figures, which are all from one and the same specimen taken six days after intraperitoneal infection, show that the parasite encircles the erythrocyte, which gradually becomes enclosed in its protoplasm, probably in a sort of vacuole which develops between the two nuclei. The erythrocyte becomes more or less disintegrated and gradually reduced in size; its characteristic staining remains for a long time recognizable, but the final result is a vacuole with homogeneous, almost unstained contents, between trophonucleus and kinetonucleus.

It is not probable that this phenomenon is of common occurrence; as far as I am aware, it has never before been described. It may, however, be mentioned that in a paper by Carini (1910, *Ann. Inst. Pasteur*, XXIV. 143–151), describing intracorpuscular forms of various trypanosomes, two figures (29 and 30) might perhaps more correctly be interpreted as showing digestion of erythrocytes. It is likewise

Harald Seidelin, pinx. University Press Cambridge.

possible that the vacuoles observed in various trypanosomes, between the two nuclei, may be of a similar nature. For the general physiology and biology of the protozoa the occurrence of such a process, although it may be an exceptional one, is obviously of considerable interest. In the case of trypanosomes, the ordinary mode of nutrition is believed to be that of diffusion through the superficial membrane, but the observations here recorded show a certain resemblance to the conditions obtaining in the case of amoebae and many other protozoa. The occasional ingestion of such large elements as erythrocytes suggests that the parasites may perhaps also ingest smaller particles, a process which it would be difficult to observe. The individuals concerned in the digestion of erythrocytes show as a rule somewhat prominent (volutin) granules in their protoplasm, but it is not clear whether there is any relationship between the two phenomena.

I am indebted to Dr A. Connal, Director of the Yaba Institute, for the facilities afforded me.

EXPLANATION OF PLATE XVIII.

Giemsa stain. Zeiss' apochr. imm. 3 mm., comp. oc. 12. Abbe's drawing apparatus. Magnification 1300.

Figs. 1–3. Ingestion of erythrocytes by trypanosomes.
Figs. 4–11. Various stages of digestion.
Fig. 12. Trypanosome containing vacuole.

ABDERHALDEN'S REACTION AND ITS APPLICATION IN CERTAIN PROTOZOAL INFECTIONS.

By EDWARD HINDLE AND LAJOS GÓZONY.

(*From the Quick Laboratory, Cambridge.*)

Introduction.

It has been known for some time that the injection of various albuminoid materials into the circulation of experimental animals, is followed by the production of antibodies in their blood, which have a neutralizing, coagulating, or dissolving action upon the material, or materials, which called forth their production. The applications of this discovery have been of the highest importance in the prevention of disease, for by the injection of certain species of bacteria into the blood-stream, antibodies can be produced, which are able to protect the body against the attacks of these organisms, as they are dissolved as soon as they enter the system. On the other hand, the antibodies do not always completely dissolve the substances against which they have been formed, and in this way partially destroyed substances may be produced in the blood-stream, and these sometimes have a toxic action upon the organism. This phenomenon is well known under the term anaphylaxis.

Not only bacteria, and other cells foreign to any particular vertebrate, are able to cause the formation of antibodies, but also the cells of its own body, if they are introduced into the blood circulation.

Weichardt (1902) has shown that in cases of eclampsia the blood-serum contains antibodies which dissolve syncitium cells. Moreover, he interpreted the symptoms of eclampsia as the result of the contents of these cells being liberated and having a toxic effect upon the body.

Naturally is it not the cells themselves, but the albumin of the cells, which is the antigen that causes the formation of antibodies.

Five years ago one of the authors (Gózony, 1909) showed that the serum of women suffering from eclampsia is toxic, and that this serum

also contains antibodies which are able to produce poisonous substances when mixed with amniotic fluid, which, of course, contains placental albumins.

Certain serologists hold the belief that these antibodies, which are capable of dissolving cells and destroying albumins, really act like digestive ferments, but Abderhalden was the first author to show definitely that the injection of foreign substances into the blood may be followed by the production of ferments which dissolve them. Previously, however, Wohlgemuth had shown that the injection of amylum into the blood of a dog was followed by a considerable rise in the amylase content of the serum.

Abderhalden's first experiments were made with dextrose. When he injected this substance intravenously into a dog, after an interval of two days the dog's serum was able to decompose dextrose, this decomposition being observed by means of the polariscope. After a few days, however, the serum lost this power.

Similar results were obtained by injecting silk-peptone into a dog, but the serum of this animal dissolved not only silk-peptone, but also albumose and other kinds of peptone. In other words the proteolytic ferments produced by the organism were not specific in this case.

It was a matter of some surprise, therefore, when Abderhalden discovered that the serum of pregnant women contains proteolytic ferments which are very specific in their action, dissolving only the peptones and albumins contained in placental tissues. These ferments do not destroy any other kinds of peptones and, moreover, are only found in the serum of pregnant animals.

In order to discover the presence of proteolytic ferments in the serum of any particular species, two methods have been employed.

(1) The more exact method depends upon the fact that polypeptids produce optically active solutions, which rotate the plane of polarised light to the left. Accordingly, a solution is prepared of the polypeptids from the placenta, or other organ to be tested, and mixed with the serum containing the ferments. Then the optical activity of this mixture is measured at once by means of a polariscope, and after two or three hours measured again. If the serum contains any ferment having an action upon the polypeptids employed, there will be an alteration in the optical activity of the mixture and this can be measured very exactly. In the case of pregnant women it is found that their serum, when mixed with polypeptids from a placenta, produce a decided change in the optical activity of the solution. The only objections

to this method are the technical difficulties involved in measuring the rotation of the light in the solutions employed, and it is only capable of application in specially equipped laboratories.

(2) The second method depends upon the fact that the molecules of albumins and polypeptids are too large to pass through an animal membrane, but the pores of the latter are not sufficiently fine to prevent the passage of peptones.

In order to employ this dialysing method, a piece of the organ to be employed is mixed with the serum under examination, and the mixture put into a dialysing capsule and dialysed in distilled water for about 16 hours. The dialysate is then tested for the presence of peptones, and if these are found it is evident that the serum must contain some proteolytic ferment, or ferments, acting upon the particular organ employed. In applying this method to the diagnosis of pregnancy, the serum of the woman to be examined is mixed with a piece of placental tissue, the mixture dialysed for 16 hours, after which the dialysate is examined for peptones, the presence of the latter denoting that the woman is pregnant.

Up to the present time more than 3000 tests have been made by this method and in every case a positive reaction was found to be a sure sign of pregnancy. In the Quick Laboratory, we also have examined the blood from a number of cases, kindly supplied to us by Dr Roderick, and obtained uniformly positive results with the sera of pregnant women.

In order to apply this test successfully, however, it is necessary to work very exactly, as there are many sources of error.

The serum to be tested must be quite clear and without any trace of haemoglobin. It is best to take the blood from a vein by means of a dry needle, and to collect it in centrifugal tubes. The blood is then left at room temperature for three to six hours, in order to let the clot separate out, and afterwards the serum is well centrifuged in order to remove all red blood corpuscles. If any of the latter are left in the serum they are dissolved during the dialysis, and as a result the haemoglobin passes through into the dialysate and interferes with the reaction.

The blood should be collected before meals, preferably before breakfast, as during the process of digestion the serum always contains more or less peptone. For this reason when performing an experiment it is always necessary to make a control employing serum alone, to see whether it contains any dialysable substances which give the Ninhydrin reaction.

The placenta (or other organs) to be used for this reaction must be carefully prepared in order to remove all peptones and other soluble materials. First, all the connective tissue and clotted blood are removed, and afterwards the organ is cut up into little pieces about 1 cm. across. These are then placed into 0·9 per cent. NaCl solution, and pressed between the fingers in order to squeeze out all the blood. The salt solution is changed until no more blood comes out of the fragments. The latter are then washed in tap water for 10 to 15 minutes, and afterwards boiled in sterilized distilled water containing five to six drops of glacial acetic acid in each 100 c.c. They are then rinsed in cold water and again boiled in distilled water—without the addition of acetic acid. When the fragments have been boiled five or six times in distilled water, changing the water between each boiling, there is a possibility that they may have been sufficiently well washed and the next process is to test them. For this purpose they are boiled for five minutes with about five times their mass of distilled water. Some of this water is then filtered, and to 5 c.c. of the filtrate is added 1 c.c. of a Ninhydrin solution and the mixture boiled for one minute. After boiling, the liquid is allowed to stand for about half-an-hour, and if a blue colour develops it is a sign that the tissue has not been sufficiently washed, and it is necessary to wash again with boiling water and repeat the process until no blue colour is obtained on applying this test.

By this time most of the fragments will appear white, but in the case of liver, spleen and kidney they are always a little coloured. It is better to remove any fragments which are decidedly brown or grey, as this is a sign that they contain haemoglobin.

It is most important that no blood should be left in the organs, as the serum often contains ferments having a digestive action upon the red blood corpuscles. The serum of quite 50 per cent. of horses and cows contains peptolytic ferments that dissolve the red blood corpuscles. If any organ therefore still contains red blood corpuscles, a positive reaction with serum does not necessarily indicate that any organ destruction has taken place. For this reason it is advisable, before using the prepared organ fragments for any reaction, to test them by adding some normal serum and then dialysing in the usual way. The presence of peptones in the dialysate, indicated by the Ninhydrin reaction, is a sure sign that the organs have not been sufficiently well washed.

Any pieces of organ prepared in the above manner may be kept indefinitely in sterilized distilled water, containing plenty of chloroform, with a layer of toluol on the surface of the water. Before using any

such organs, however, it is necessary to test them again by boiling with distilled water, filtering, and testing the filtrate with Ninhydrin.

Technique.

The dialysing capsules most convenient for this reaction are those supplied by Schleicher and Schüll, known as No. 579 A.

These capsules must be tested before being used in order to find out whether they are impermeable to albumin, but still allow peptone to pass through without any difficulty. It will generally be found that a certain percentage of the capsules are defective in respect to permeability, some being too porous and allowing albumin to pass through, whilst others are too dense and resist the free diffusion of peptones. In order to test them the capsules are first softened by soaking in distilled water. Then into each capsule is put 2·5 c.c. of a 5 per cent. solution of egg albumin, prepared from the white of a fresh egg. Each capsule is then placed in a small Erlenmeyer flask, or a bottle, containing 20 c.c. of distilled water. The flask should have a neck sufficiently wide to receive the capsule readily, the neck being slightly longer than the capsule.

Toluol is then poured into the capsule and also upon the water in the flask, until it forms a layer about 1 cm. thick. The flasks, with their contained capsules, are then incubated at 37° C. and allowed to stand for about 16 hours.

The dialysate is then tested for the presence of albumin either by means of the Biuret reaction, or by adding sulpho-salicylic acid. In the former case, 10 c.c. of the dialysate is mixed in a test-tube with 2·5 c.c. of a 33 per cent. solution of caustic soda. Then, by means of a pipette, 1 c.c. of a 0·2 per cent. solution of copper sulphate is carefully run on to the surface of the mixture and if a violet ring is formed at the junction of the two liquids, the presence of albumin is denoted. Any capsules which are thus shown to be permeable to albumin should be discarded. The dialysate may also be tested by adding to 10 c.c. of the dialysate, 1 c.c. of a 33 per cent. solution of sulpho-salicylic acid, the presence of albumin being indicated by the liquid developing an opalescent or milky appearance.

The capsules found to be impermeable to albumin are washed in running water for 24 hours, and then dipped in boiling distilled water for half-a-minute. They are then tested for permeability to peptone.

For this purpose into each capsule is placed 2·5 c.c. of a 1 per cent. solution of silk peptone and the fluid dialysed for 16 hours in 20 c.c.

distilled water at 37° C., in exactly the same way as the albumin, the liquid both within and without the capsule being covered with a layer of toluol as before. From each flask, 10 c.c. of the dialysate is then mixed with 0·2 c.c. of a 1 per cent. solution of Ninhydrin, and the mixture boiled in a test-tube for one minute. In every case a deep blue colour should be developed and if the intensity of the shade is not the same in all the tubes it is a sign that the capsules are not of equal permeability. Those capsules which only give a slight reaction must be discarded, as they are too dense to be employed.

The capsules which have passed both these tests are washed once more in running water for 24 hours, then in boiling distilled water for 30 seconds, and may be preserved in chloroform-water covered with a layer of toluol.

When making a test, about 0·5 gm. of the prepared organ is placed in a capsule (*a*) and mixed with either 0·5, 1·0, or 1·5 c.c. of the serum to be examined. In another capsule (*b*) is placed an equal volume of serum alone, whilst in a third capsule (*c*) is placed an equal volume of *inactivated* serum[1] mixed with 0·5 gm. of the prepared organ. Each capsule is then inserted in a flask containing 20 c.c. of sterilized distilled water and the surface of all the liquids covered with a layer of toluol. Then dialysis is allowed to continue at 37° C. for about 16 hours.

The dialysates are tested for the presence of peptones by means of the Ninhydrin reaction, 0·2 c.c. of a 1 per cent. solution of Ninhydrin being added to 10 c.c. of each dialysate and the mixture boiled for exactly one minute. If the controls, *b* and *c*, both remain uncoloured whilst the dialysate of organ and serum gives a positive reaction, indicated by the development of a blue colour, it is a sign that the serum contains ferments which can digest the substance of the particular organ employed. If the controls are slightly coloured, whilst the organ and serum is deeply coloured, so that there is a marked difference between them, the reaction is also positive ; on the other hand, should there be a slight difference between the colour of the organ and serum and the controls it is better to repeat the test employing only 0·5 or 1 c.c. serum, in order to bring out any differences more distinctly. After testing it is well to wash the capsules at once for 24 hours in running water followed by 30 seconds boiling in distilled water, as in this way they can be used four or five times before becoming too dense.

[1] Inactivated by heating at 60° C. for half-an-hour.

Application to the study of disease.

The application of this reaction for the diagnosis of pregnancy is sufficiently well known, but it may also be employed for the study of disease. If a certain organ of the body is diseased, as a result of the degeneration of its tissue, some of its albumins will enter the blood and induce the formation of ferments which act on these particular albumins. These ferments seem to be specific in their action since they only digest those albumins which called forth their production. For example, if the liver of a particular animal is diseased, liver albumins are set free in the blood circulation by the breaking down of liver-cells and the subsequent liberation of their contents. Specific ferments would then be produced in the blood, which can digest these liver albumins, and by collecting some of the serum from such an animal and mixing it with liver tissue from the same species, prepared in the manner described above, it is possible to detect the presence of these ferments, as the liver substance is broken down into dialysable peptones, or amino-acids.

When an animal is infected with any pathogenic micro-organisms, in most cases ferments are produced which digest them, and thus it is possible to detect what kind of micro-organism is infecting any particular animal by the changes in its blood.

This reaction seems likely to be of value in the diagnosis of protozoal infections, because in these cases the ordinary immunity reactions are very variable and uncertain. In the case of spirochaetosis, trypanosomiasis and sarcosporidiosis, one of the authors (Gózony, 1914) has obtained very favourable results by the application of Abderhalden's reaction, and accordingly we have examined the blood from animals suffering from another class of protozoal infection, in order to see whether similar results could be obtained in these cases.

East Coast Fever (*Theileria parva*).

Experiments were made with the serum from calves infected with East Coast Fever. The animals were infected by feeding infected ticks (*Rhipicephalus appendiculatus*) on them. After an incubation period of about eleven to twelve days a calf shows a rise in temperature; about five days later parasites appear in the peripheral circulation, and

gradually increase in number until the animal dies, the total duration of the disease from first putting on infected ticks to the death of the animal rarely exceeding one month.

The pathological lesions observed at autopsy in a calf dead of East Coast Fever are relatively insignificant, practically none of the organs being markedly affected, and therefore this seemed a very suitable disease to study by means of Abderhalden's method.

Experiment 1. On March 7th, 1914, 50 infected nymphs of *Rhipicephalus appendiculatus* were placed on a calf. On March 19th, it first showed febrile symptoms, parasites appeared in the peripheral blood five days later, and finally, on April 2nd. it was killed when moribund. On March 27th, when 12·8 per cent. of the corpuscles contained one or more parasites, a small quantity of the calf's blood was collected and the serum tested for the presence of ferments against the following organs from a normal calf.

In every case 1·5 c.c. of the serum from the infected calf was mixed with about 0·5 gm. of the organ (prepared as described above) and the mixture dialysed for about 16 hours at 37° C.

As a control the serum from a normal calf was mixed with corresponding amounts of the same organs.

The results were as follows, a positive or negative reaction being indicated by a + or – sign respectively:

Organ employed	+ 1·5 c.c. infected serum	+ 1·5 c.c. normal serum
Brain	+	–
Kidney	–	–
Liver	+	–
Lymphatic gland	+	–
Lung	+	–
Pancreas	+	–
Spleen	+	–
Suprarenal gland	+	–
Thyroid ,,	+	–
Thymus ,,	+	–
Control (serum alone)	–	–
Control ,,	–	–

On April 2nd, when the calf was killed, all the organs were found to contain infarcts with *Theileria parva* present. Moreover, quite 40 per cent. of the red blood corpuscles contained one or more parasites. The organs of this calf were prepared in the usual manner and employed for another series of tests with the calf's own serum.

The results were as follows :

Organ employed	+1·5 c.c. infected serum	+inactive infected serum
Infected Brain	+	–
„ Diaphragm	+	–
„ Heart muscle	+	–
„ Lymphatic gland	+	–
„ Lung	–	–
„ Muscle	+	–
„ Pancreas	+	–
„ Spleen	+	–
„ Suprarenal gland	+	–
„ Thymus „	+	–
„ Thyroid „	+	–
Control (serum alone)	–	–
„ „	–	–

In addition, the red blood corpuscles were isolated from about one litre of this calf's blood and after being well washed with normal salt solution were cytolized by means of digitoxin in order to isolate the parasites. After cytolysis was complete the parasites were washed free from haemoglobin and part of them were coagulated by mixing with egg albumin and boiling. The resulting coagulum was mixed with infected serum and dialysed, when a strongly positive reaction was obtained with the dialysate.

From the above results it will be seen that the blood of a calf infected with East Coast Fever, contains ferments which can dissolve the albumin of almost all organs, as well as the isolated parasites.

Agglutination and complement fixation tests were also tried with other lots of the isolated parasites, but with uniformly negative results.

Experiment 2. On April 30th, 100 infected nymphs (*R. appendiculatus*) were fed on a calf. On May 12th it first showed a rise in temperature and was bled and its serum tested for the presence of ferments. Six days later (May 18th), one day after parasites were first seen in the peripheral circulation, it was again bled and the serum tested as before, whilst yet a third series of tests were made with the blood of this animal on May 25th, when at least 30 per cent. of the corpuscles were infected. The animal died of East Coast Fever the same night.

In every case 1 c.c. of the calf's serum was mixed with about 0·5 gm. of the organ to be employed, and in most cases the organs used had been obtained from the calf of Experiment 1, and therefore contained parasites.

As a control, some of the organs were mixed with inactivated serum as well as active.

The results were as follows, all three series of tests being tabulated together for the sake of comparison :

Organ employed	Blood collected from calf, May 14th		Blood collected from calf, May 20th		Blood collected from calf, May 28th	
	+1 c.c. active serum	+1 c.c. inactive serum	+1 c.c. active serum	+1 c.c. inactive serum	+1 c.c. active serum	+1 c.c. inactive serum
Infected Brain	–		–		–	
„ Diaphragm	–		–			
„ Heart	–		–		+	
„ Kidney	–		–		+	
„ Liver	–	–	–	–	+	–
„ Lymphatic gland	–		+		+	
„ Lung	–	–	–		+	
„ Muscle	–		–		–	
„ Pancreas	–		–		+	
„ Spleen	–	–	–		+	
„ Suprarenal gland	–		+		+	
„ Thymus „	–		–		–	
„ Thyroid „	–		–		+	
Normal Liver					+	–
„ Kidney					+	–
Isolated parasites			+	–		
Control (serum alone)	–	–	–	–	–	–
„ „	–	–	–	–	–	–

When calf 2 first showed febrile symptoms, no ferments could be detected in its blood, but six days later the serum gave distinct reactions with lymphatic and suprarenal glands, and isolated parasites. The result is in accordance with what is known of the pathology of East Coast Fever, as the first organs to be affected are the lymphatic glands, where the so-called " blue bodies," a stage in the life-cycle of *Theileria parva*, are found before the parasite can be detected in the peripheral circulation.

After another five days, when the animal was very heavily infected, all the organs, with the exception of the brain and thymus, gave positive reactions, thus showing that destruction of tissue was taking place in all these organs.

Babesia canis.

Two experiments were made with the serum of dogs infected with *Babesia canis*, but owing to the haemolysis produced by the parasites it was only possible to obtain satisfactory reactions when the blood was taken at early stages of the disease. During the later stages

the serum contained so much haemoglobin that the reactions obtained in most of the experiments were positive.

Experiment 1. A dog was inoculated with infected blood on April 20th. Seven days later parasites appeared in its blood and on April 30th it was killed, and its serum and organs used for the following tests :

Organs		Reaction
Brain (from *Babesia* dog)	+ 1 c.c. infected serum	–
Liver ,,	+ 1 c.c. ,, ,,	+
Pancreas ,, ,,	+ 1 c.c. ,, ,,	–
Kidney ,, ,,	+ 1 c.c. ,, ,,	–
Lung ,, ,,	+ 1 c.c. ,, ,,	–
Heart Muscle ,,	+ 1 c.c. ,, ,,	–
Control (serum alone)	1 c.c. ,, ,,	–
,, ,,	1 c.c. ,, ,,	–

This experiment, therefore, indicates that destruction of liver tissue has already commenced, but that the other organs are as yet unaffected.

Experiment 2. A dog was inoculated with infected blood on June 8th. Five days later parasites appeared in its blood and on June 15th, when it was heavily infected, the dog was killed and its blood tested with the following organs. In order to minimise the effect of the slight amount of haemoglobin present in the serum, only 0·5 c.c. was employed in each case.

Organ		Result
Liver	+0·5 c.c. active serum	+
Brain	+0·5 c.c. ,, ,,	+
Pancreas	+0·5 c.c. ,, ,,	+
Heart	+0·5 c.c. ,, ,,	+
Lung	+0·5 c.c. ,, ,,	+
Liver	+0·5 c.c. inactivated serum	–
Heart	+0·5 c.c. ,, ,,	–
Brain	+0·5 c.c. ,, ,,	–
Control (serum alone)	0·5 c.c. active serum	–
,, ,,	0·5 c.c. ,, ,,	–

The results of this experiment suggest that at a late stage of infection not only is the liver affected, but also the other organs of the body.

In both experiments the results agree with the pathology of this disease. The liver is especially affected, as evidenced by the appearance of jaundice in the majority of infected dogs, whilst at later stages of the disease, Graham-Smith (1905) has shown that extensive lesions may be observed in practically all the organs.

SUMMARY.

The results of the above described experiments suggest that Abderhalden's reaction may be of use in the study of protozoal diseases.

In the case of East Coast Fever it is seen that in early stages of the disease only the lymphatic and suprarenal glands seem to be affected, whilst in later stages destruction of tissue takes place in practically all the organs, and this corresponds with the results of microscopical examinations of the various tissues.

Various experiments were performed with dogs infected with *Babesia canis*, but in most cases, owing to the presence of haemoglobin in the serum, the controls gave positive results as well as the organs + serum. The blood of a dog taken at the commencement of the infection was found to contain substances having a destroying action on liver tissue, but not on the other organs employed, whilst in later stages of the disease practically all the organs were affected.

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A LIST OF NEMATODE PARASITES OBSERVED IN THE ALIMENTARY CANAL OF SHEEP IN ENGLAND.

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(With Plate XIX and 4 Text-figures.)

Whilst engaged in the study of the life-histories of certain Nematode parasites of domestic animals, I have had the opportunity of examining the alimentary tracts of a number of sheep and lambs obtained from various localities in England during the last 12 months, and have thus been able to collect and identify a considerable number of the parasitic Nematodes which are found so abundantly in this situation.

Owing chiefly to the researches of Ransom in the United States, Railliet and Henry in France and Looss in Egypt, our knowledge of the round-worm parasites of sheep and other ruminants has increased considerably during recent years; little work has, however, been done on this subject in England, the larger and more conspicuous forms have received some attention, yet very few records exist of the smaller species or of those which require careful microscopical examination for their identification.

The object of the present paper is to furnish a complete list of the parasitic Nematodes observed in the digestive tract of sheep during the past year. Thanks to Ransom's very complete monograph (1911) the identification of these parasites no longer presents great difficulties; about 28 species of Nematodes, belonging to 13 genera, have been recorded from different parts of the world, of these I have met with

in England 13 species, distributed among 10 genera, that is to say nearly half of the known species.

My material was obtained from various sources; thanks to the courtesy of Mr Brailsford I was able to examine a number of sheep and lambs from the Birmingham slaughter houses, other animals were procured from farms in the neighbourhood of this city, whilst a number of interesting observations were made at Wye in Kent during a short visit to the South Eastern Agricultural College in August 1913. I should like to acknowledge here my great indebtedness to Mr H. E. Hornby of that institution for placing a quantity of his material at my disposal and for his generosity in giving me the benefit of his large experience of the parasitic diseases of sheep.

In this paper no attempt has been made to give complete descriptions of the various species observed and the commoner and better known forms are merely mentioned by name; in the case of some of the rarer and less easily identified forms short descriptions are given together with some measurements and figures, partly to supplement previous accounts, but chiefly in order that others may judge as to the accuracy of my determinations. Among such forms special attention may be called to the following:

Ostertagia trifurcata Ransom, previously known from the United States only[1]; *Cooperia oncophora* Railliet, known from cattle in Europe but not before recorded from the sheep in this country, and the three species of *Trichostrongylus* of which little seems known in England.

Glycerine mounts were found the most satisfactory for the microscopical examination of the specimens, the worms being killed in hot 70 per cent. alcohol and glycerine according to the well-known method first suggested by Looss. For the elucidation of the finer structure of the spicules it was sometimes found desirable to use a mounting medium of higher refractive index than glycerine. A convenient medium is one sometimes used by entomologists for the examination of the more delicate chitinous structures in insects, this consists of 10 parts of a saturated solution of gum arabic in cold water, 1 part of glycerine, 1 part of chloral hydrate, 1 part of glacial acetic acid and 1 part of 95 per cent. alcohol. Specimens may be transferred directly from water or alcohol into this medium which has the additional advantage of solidifying rapidly.

[1] For the first record of *Ostertagia trifurcata* in England, priority must be given to Mr T. Storrar Cave, whose note on this species, published in the present number of this *Journal* (p. 201), was received for publication before Dr Boulenger's paper.—Ed.

In the following list I have adopted the nomenclature used by Ransom, synonymies have not been given, but to the correct names of the species I have in most cases added the older and more familiar names used in the majority of zoological and veterinary text-books. For complete descriptions and synonymies of the different species the reader is referred to Ransom's monograph (1911).

Family **STRONGYLIDAE.**

Sub-Family **Strongylinae.**

1. **Bunostomum trigonocephalum** Rudolphi.

(*Uncinaria cernua* Creplin.)

This species was found on several occasions in sheep from the Birmingham slaughter houses, it occurred in the small intestine, always in small numbers.

2. **Chabertia ovina** Fabricius.

(*Sclerostomum hypostomum* Rudolphi.)

A common parasite of the large intestine found at Wye (Kent), Tenbury (Worcs.) and in sheep from the slaughterhouses, sometimes in considerable numbers.

3. **Oesophagostomum venulosum** Rudolphi.

Found occasionally in the large intestine of sheep at Birmingham and at Tenbury (Worcs.).

Sub-Family **Trichostrongylinae.**

4. **Haemonchus contortus** Rudolphi.

(*Strongylus contortus* Rudolphi.)

Common in the fourth stomach (abomasum) of sheep at Wye, Kent.

5. **Ostertagia circumcincta** Stadelmann.

(*Strongylus cervicornis* McFadyean.)

From the fourth stomach and small intestine of sheep at Wye (Kent) and Birmingham, very common in the former locality.

6. **Ostertagia trifurcata** Ransom.

Whilst recently examining a number of specimens of *Ostertagia circumcincta* collected at Wye from the fourth stomach of a lamb suffering from parasitic gastritis, I found two male individuals which, although obviously belonging to the genus *Ostertagia*, differed markedly from the males of the common species. A detailed study of the bursae and spicules showed that these worms were to be referred to the rare species *Ostertagia trifurcata* described by Ransom in 1907. This species has been met with in the United States only, in small numbers in the fourth stomach and intestine of sheep and goats, and always associated with *Ostertagia circumcincta*; the occurrence of *O. trifurcata* in sheep in England is therefore a new fact in its distribution.

The female of *O. trifurcata* was not found by Ransom; in the material collected at Wye there were, however, two *Ostertagia* females which differ in many respects from the females of *O. circumcincta* and which I take to belong to the former species. The measurements of the male specimens differ slightly from those given by Ransom (1911), I have therefore given a fairly complete description of these, as well as of the female individuals.

Males. As mentioned above only two males were obtained from the material collected at Wye, these measured 6 and 6·8 mm. in length, with maximum breadths of 85 and 98 μ, respectively, in the region of the body just in front of the bursa.

The longitudinal lines of the cuticle are well marked in both specimens.

The diameter of the head is 16 μ in the larger individual, 20 μ in the smaller, the latter has the cuticle slightly dilated in the cephalic region. The breadth of the body at the posterior extremity of the oesophagus is 60 μ.

The oesophagus has a length of 480–490 μ with a maximum thickness of 30–48 μ posteriorly.

The nerve ring and the cervical papillae were observed in the smaller specimen only, their distances from the anterior end of the body being 210 μ and 260 μ, respectively.

The genital bursae are somewhat folded in both individuals, when spread out they must have had a breadth of between 280 and 300 μ. The prebursal papillae are conspicuous.

The arrangement of the bursal rays is similar to that described

by Ransom and is shown in the accompanying figure (Pl. XIX, fig. 2). The latero-ventral rays are somewhat thicker than the other rays. The stem of the dorsal ray measures 35–40 μ, its main branches 27–28 μ; each of the latter bears a short inner branch close to its distal extremity, whilst a slightly longer branch is given off more proximally, this outer branch projects backwards, not forwards, as in the specimen figured by Ransom (1911).

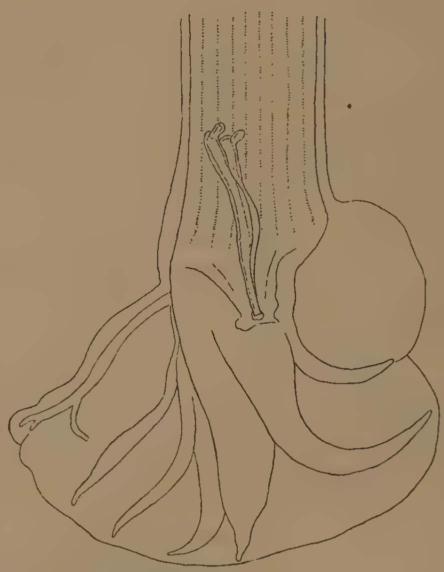

Fig. 1. *Ostertagia trifurcata* Ransom. Male and female. ×15.
Fig. 2. *Cooperia oncophora* Railliet. Posterior end of body of male, viewed from right side. ×100.

The two spicules are approximately equal in size, measuring 150 μ in length in the smaller individual, 160 μ in the larger; each spicule is divided into three processes posteriorly, the principal one long, thick and truncated distally, the other two shorter and terminating in fine points.

The gubernacula measure 62 μ and 65 μ in length, they are broadest in the middle and taper towards both extremities; in one specimen

the posterior narrow region is not straight but follows a somewhat sinuous course (Pl. XIX, fig. 4).

Females. The two female individuals measure 7·5 and 6·3 mm. in length; the smaller specimen is immature and the measurements given below are taken chiefly from the larger one.

The body is thickest in the region of the vulva where it has a width of 100 μ, the diameter at the base of the oesophagus is 62 μ, at the anus 40 μ.

The longitudinal ridges of the cuticle show up clearly and have the finely beaded appearance noticed by Ransom in the type specimens.

The oesophagus measures 500 μ in length with a maximum breadth of 40 μ posteriorly.

The anus is about 110 μ from the tip of the tail, the latter being slender and gradually attenuated, ending in a rounded tip, devoid of the annular striations so characteristic of *Ostertagia circumcincta*.

The vulva is a transverse, naked slit, situated 1·15 mm. from the posterior end of the body, it is not protected by a projecting flap of cuticle.

The muscular portions of the ovijectors have a length of 300 μ, ncluding the sphincters.

The eggs in the uterus measure 85–95 μ long by 40–48 μ wide, they are very similar to those of *O. circumcincta*.

7. **Cooperia oncophora** Railliet.

(*Strongylus ventricosus* Rudolphi [in part].)

A number of specimens of this worm were obtained on one occasion from the small intestine of a sheep received from the Birmingham abattoirs.

C. oncophora has been found in cattle and sheep in the United States, and in cattle in different parts of Europe; this is I believe the first record of its occurrence in sheep in England.

The specimens collected in Birmingham agree in every respect with those described by Ransom in his monograph. The males measure 6–8·8 mm. in length by 250–300 μ in width in the thickened region at the base of the bursa. The spicules are 270–300 μ long and are of simple structure. The arrangement of the bursal rays is shown in Fig. 2.

The females have a length of 8–8·5 mm., the body is thickest in the region of the vulva, where the cuticle is much dilated. The vulva is a transverse slit, with prominent lips, situated about 2 mm. from the

posterior end of the body. The tail is slender and its posterior portion marked with faint annular striations. The eggs in the uterus measure 60–80 μ long by 30–40 μ wide.

8. **Nematodirus filicollis** Rudolphi.

(*Strongylus filicollis* Rudolphi.)

This species was found to be one of the commonest parasites of the alimentary tract, occurring in large numbers in the small intestine of sheep and lambs both at Birmingham and Wye.

According to Railliet and Henry (1912), there has been much confusion between this species and the closely allied *N. spathiger* Railliet, the worms described by Ransom belonging to the latter species. All the specimens of *Nematodirus* which I have examined belong to the species *filicollis*, differing from *N. spathiger* in the absence of the dorsal lobules of the bursa and in the shape of the terminal membrane of the spicules which is lanceolate, not spatulate.

9. **Trichostrongylus instabilis** Railliet.

I have only met with a single specimen of this worm, a male 4·2 mm. long found together with *T. extenuatus* in the intestine of a lamb at Tenbury. The spicules measure 130 μ in length and agree very closely with those figured by Looss (1905) and Ransom (1911). (Cf. Fig. 3.)

10. **Trichostrongylus vitrinus** Looss.

A common parasite of the small intestine and fourth stomach of sheep at Birmingham and Wye. I do not know of any previous records of its occurrence in England, where it has probably been confused with *T. extenuatus*. From the other species of *Trichostrongylus* it is easily distinguished by the large size of the bursa and spicules, the latter being more than 160 μ long. The shape of the spicules is also very characteristic, ending in sharp points without hook-like projections (Fig. 4).

Some of my specimens of *T. vitrinus* are somewhat larger than those described by Looss and Ransom, the males measuring 4·5–6·3 mm. in length, the females 5·5–7·2 mm. The male bursa has a width of 300–350 μ, whilst the spicules vary between 160 and 180 μ. The

branches of the dorsal ray are usually split at their distal extremities, not simple as in the specimens previously described.

In both sexes the cuticle is often much dilated just behind the head, in this respect resembling *T. retortaeformis* Zeder. I am inclined

Fig. 3. *Trichostrongylus instabilis* Railliet. Spicules. ×450.
Fig. 4. *Trichostrongylus vitrinus* Looss. Spicules. ×450.

to believe that this is not a post-mortem phenomenon, having observed it in many living specimens of the latter species.

The females agree well with those described by Looss (1905). The eggs measure 80–95 μ by 40–52 μ.

11. **Trichostrongylus extenuatus** Railliet.

(*Strongylus gracilis* McFadyean.)

This species is easily recognised by the marked dissimilarity in shape and size of the two spicules. I have collected specimens from Wye and Tenbury.

Family ANGIOSTOMIDAE.

12. Strongyloides papillosus Wedl.

(*Rhabdonema longus* Grassi and Segrè.)

I have met with this species on one occasion only, a single specimen was found in the small intestine of a sheep from the Birmingham slaughter houses. The specimen measured 5·4 mm. in length by 55 μ in maximum thickness. The thin-shelled eggs were 48–50 μ long by 20–23 μ wide.

Family TRICHINELLIDAE.

13. Trichuris ovis Abildgaard.

(*Trichocephalus affinis* Rudolphi.)

This is perhaps the commonest of all the parasites of the alimentary tract. I have found specimens in the large intestine of almost every sheep I have examined. *T. ovis* usually occurs in small numbers; on one occasion, however, I collected more than 250 of these worms from a single intestine.

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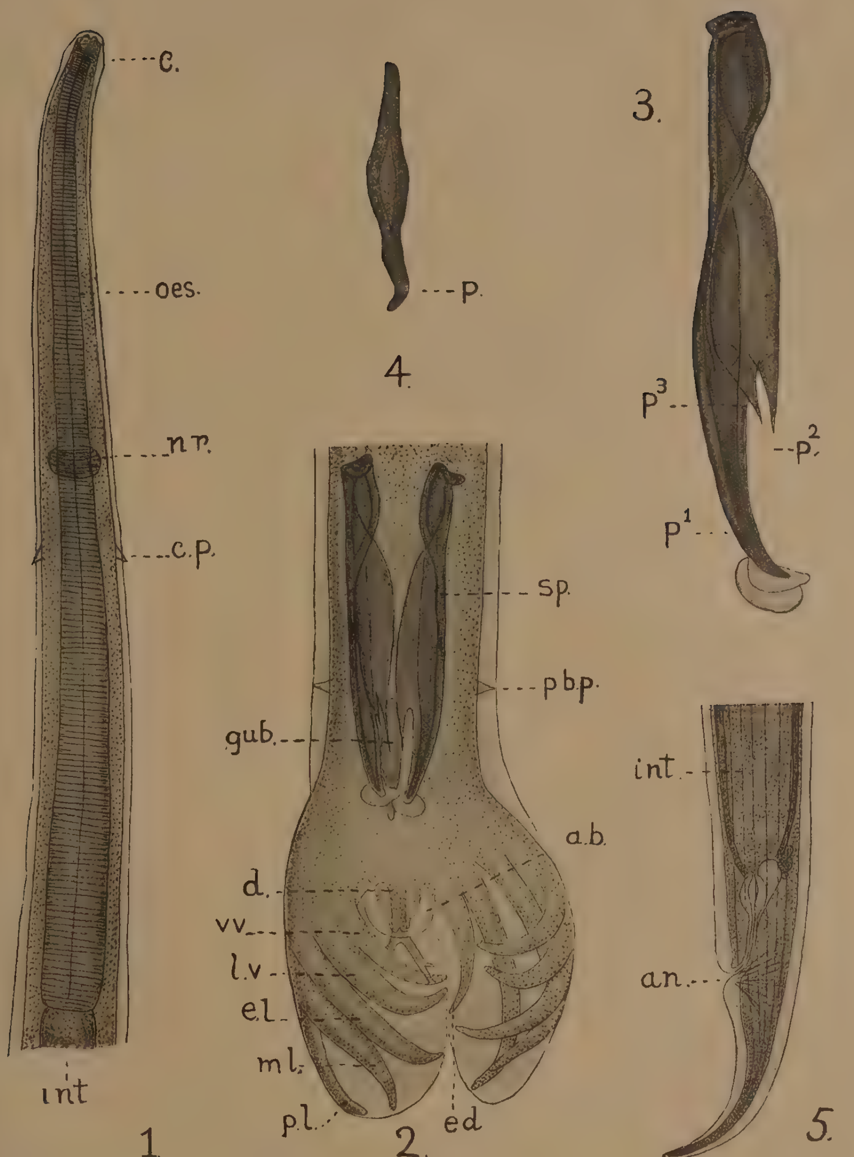
EXPLANATION OF PLATE XIX.

Ostertagia trifurcata Ransom.

Fig. 1. Male; anterior extremity. ×300 diameters.
Fig. 2. Male; posterior end of the body with the spicules and bursa. ×300 diameters.
Fig. 3. Male; right spicule, ventral view. ×600 diameters.
Fig. 4. Male; gubernaculum, ventral view. ×600 diameters.
Fig. 5. Female; posterior end of the body. ×300 diameters.



C. L. B. del.

Ostertagia trifurcata.

INDEX TO LETTERING.

a.b.	accessory bursal membrane.
an.	anus.
c.	head.
c.p.	cervical papilla.
d.	dorsal ray of the bursa.
e.d.	externo-dorsal ray.
e.l.	externo-lateral ray.
gub.	gubernaculum.
int.	intestine.
l.v.	latero-ventral ray of the bursa.
m.l.	medio-lateral ray.
n.r.	nerve ring.
oes.	oesophagus.
p.	posterior narrow end of gubernaculum.
p^1.—p^3.	the three processes of the spicule.
p.b.p.	prebursal papilla.
p.l.	postero-lateral ray of the bursa.
sp.	spicule.
v.v.	ventro-ventral ray of the bursa.

TICK ABNORMALITIES.

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With 11 Text-figures.)

In a paper published with Warburton in 1909 (*Parasitology*, II. 70–76, Figs. 17–26), I described and figured a number of abnormalities observed in ticks of which the most remarkable were two specimens, *Boophilus decoloratus* ♂ and *Rhipicephalus sanguineus* ♂ (Figs. 22, 25 and 26), showing two ani together with a duplication of the adanal shields; in the *Rhipicephalus* duplication was more pronounced in that a subcircular spiracle was present posteriorly in the median line; the *Boophilus* exhibited two caudal spines. The other specimens figured were *Hyalomma aegyptium* ♂ and *Boophilus australis* ♂ (Figs. 18 and 21) which showed underdeveloped adanal plates on the right side. All of these specimens were found attached to a host.

In the course of the last five years, during which some thousands of ticks have passed through our hands, a number of abnormalities have been encountered, of which 11 are herewith described and figured since they are sufficiently remarkable to be recorded. All of the figures were drawn by me with the aid of a Camera lucida and Zeiss binocular dissecting microscope.

Specimen 1.

(Fig. 1.) *Rhipicephalus appendiculatus* Neumann, 1908, ♂ (N. 948), from Cape Colony, received XII. 1909 from Mr C. P. Lounsbury, who kindly presented me with the specimen. The posterior portion of the body shows duplication, the creature possessing two ani and two sets of adanal shields; it somewhat resembles the two monstrosities already

referred to as having been described in 1909. A normal ♂ is illustrated in *Parasitology*, VI. 200, Fig. 3 *a*.

Fig. 1. *Rhipicephalus appendiculatus* ♂. Monstrosity.

Specimen 2.

(Fig. 2.) *Ixodes caledonicus* Nuttall, 1910, ♀ (N. 1542), captured, together with other specimens, in a *dovecote* at Dunipace, Stirlingshire, Scotland, 25. v. 1911, by Mr W. Evans, of Edinburgh. This specimen, which had fed on pigeons, is abnormal in respect to the anal grooves

Fig. 2. *Ixodes caledonicus* ♀. Abnormal anal grooves. The left posterior portion of the body is underdeveloped.

which form a closed circle around the anus as in normal *Ixodes rasus* Neumann, 1899. The left side of the body is somewhat under-developed. In normal specimens of *I. caledonicus* ♀ the anal grooves run backward in an almost parallel manner and are not joined posteriorly,

as will be seen by reference to *Parasitology*, III. 408, Fig. 1. This abnormality suggests that the circular anal grooves may be a primitive character in *Ixodes*. Anal grooves converging to a point behind occur in *I. ugandanus* Neumann, 1906, *I. australiensis* Nn. 1904, and *I. holocyclus* Nn. 1899, whilst horse-shoe-shaped anal grooves occur in the African forms *I. lunulatus* Nn. 1907, *I. schillingsi* Nn. 1901, *I. pilosus* Koch, 1844, *I. daveyi* Nuttall, 1910, and *I. oldi* Nuttall, 1910. The horse-shoe-shaped anal grooves may be regarded as intermediate between the closed grooves above mentioned and those that run backward without converging posteriorly, *i.e.* the parallel type of anal grooves usually seen in *Ixodes* ♀.

Specimen 3.

(Fig. 3.) *Haemaphysalis leachi* (Audouin, 1827), ♂ (N. 1085, from Indian Museum 5994/10), found on *Canis aureus* in the Museum Compound, Calcutta, shows no festoons either dorsally or ventrally on

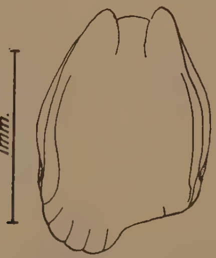


Fig. 3. *Haemaphysalis leachi* (Indian form) ♂. Abnormal: absence of festoons on the right side.

the right side, which appears atrophied. (The short body is characteristic of the Indian form of this species.) A normal ♂ is illustrated in *Parasitology*, VI. 308, Fig. 5.

Specimen 4.

(Fig. 4.) *Rhipicephalus sanguineus* (Latreille, 1804), ♂ (N. 1210, from Indian Mus., 810*a*/10), found on a *dog*, on the Puri-Orissa Coast, India, shows underdeveloped adanal shields on the right side, together with a corresponding partial absence of the festoons. A normal ♂ is illustrated in *Parasitology*, VI. 74, Figs. 23–24.

Fig. 4. *Rhipicephalus sanguineus* ♂. Abnormal: underdeveloped festoons and adanal shields on the right side.

Specimen 5.

(Fig. 5.) *Rhipicephalus sanguineus* ♂ (N. 1683), found on *Vulpes leucopus*, at Bhuj, Central India, 1911, by Mr C. A. Crump. The specimen shows very weakly chitinized adanal shields which do not protrude from the surface of the abdomen; the right side is underdeveloped, bears but three legs (leg I absent) and has a smaller adanal shield than on the opposite side.

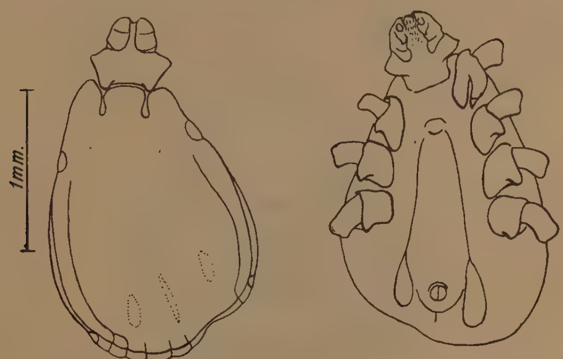


Fig. 5. *Rhipicephalus sanguineus* ♂. Abnormal: underdeveloped right side bearing but three legs, etc.

Specimen 6.

(Fig. 6.) *Rhipicephalus longiceps* Warburton, 1912, ♂ (N. 351, type lot), found on a *Klipspringerbok*, at Benguella, Angola, West Africa, by Dr F. C. Wellman, and received by us in XII. 1907. Viewed

dorsally, the caudal protuberance is accompanied by two rounded protrusions, the festoons are somewhat underdeveloped. Viewed

Fig. 6. *Rhipicephalus longiceps* ♂. Abnormal: protrusions above the caudal process; underdeveloped adanal shields of the left side.

ventrally, the adanal shield on the left side is reduced to a small projecting chitinized point. A normal ♂ is illustrated by Warburton in *Parasitology*, v. 13, Fig. 6.

Specimen 7.

(Fig. 7.) *Amblyomma marmoreum* Koch, 1844, ♂ (N. 240), from Khartoum, Soudan, 1908; sent by Dr A. Balfour. This example was found by Mr L. E. Robinson whilst examining our numerous specimens of this species. The abnormality here figured is remarkable in that

Fig. 7. *Amblyomma marmoreum* ♂. Abnormal: irregularities of the festoons.

there are but eight festoons present and that they show great irregularities and partial fusion, especially on the right side. The ventral festoons correspond with those seen dorsally. Normally, there are 11 festoons present as in other ticks which possess festoons.

Specimen 8.

(Fig. 8.) *Rhipicephalus pulchellus* (Gerstäcker, 1873), ♂ (N. 1446), found on a *rhinoceros* at Yatta Plains, Ukamba Province, British East Africa, 6. VIII. 1910, by Mr S. W. J. Scholefield. The specimen shows anomalies in respect to the legs, shields and festoons, the latter, as

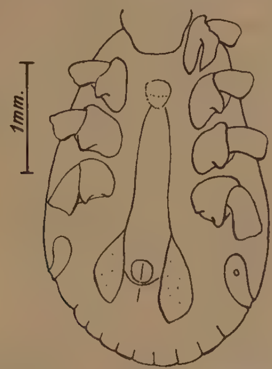


Fig. 8. *Rhipicephalus pulchellus* ♂. Abnormal: underdevelopment of the right side, which bears but three legs, small spiracle and adanal shield.

well as the spiracles, being smaller on the right than on the left side. There are but three legs on the right side (leg I is missing). The dorsum (not figured) shows an asymmetrical postero-median spot. A normal ♂ is illustrated, by Cunliffe, in *Parasitology*, VI. 210, Fig. 4.

Specimen 9.

(Fig. 9.) *Boophilus annulatus* (Say, 1821), ♂ (N. 1596), received

Fig. 9. *Boophilus annulatus* ♂. Abnormal: underdeveloped left side which bears but two legs, the adanal shields being reduced in size.

from Boston, Massachusetts, U.S.A., possesses but two legs on the left side (legs II and IV are missing); as in Fig. 5, the tick's body curves to the underdeveloped side. The left adanal shields are underdeveloped.

Specimen 10.

(Fig. 10.) *Amblyomma sublaeve* Neumann, 1899, nymph (N. 2955), found on *Manis javanica*, at Karkecet, Burma, by Fea (presented by Prof. R. Gestro, of the Genoa Museum, the specimen being one of a lot of specimens of the same species from Supino's collection labelled

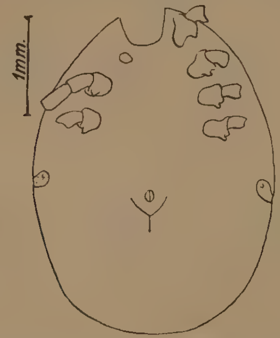


Fig. 10. *Amblyomma sublaeve* nymph. Abnormal: underdeveloped right side bearing but two legs and a small chitinous plaque in lieu of a third leg.

Rhipicephalus javanensis). The abnormality consists in there being but two legs on the right side. Legs I and II (or III ?) are missing, and one or the other is perhaps represented by a small chitinized plaque situated in the place left vacant by the absent legs.

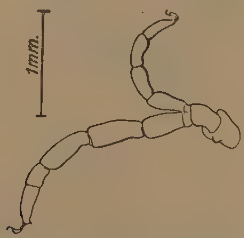


Fig. 11. *Hyalomma aegyptium* ♂. Abnormal: supernumerary leg springing from trochanter II of the right side.

Specimen 11.

(Fig. 11.) *Hyalomma aegyptium* (Linnaeus, 1758), ♂ (N. 2150), from a *bullock*, Lahore, Punjab, India, IV. 1913; sent by Major J. N. Mathers with other specimens. This is the first example of the kind I have encountered in ticks. The specimen possesses a small supernumerary leg arising anteriorly from the trochanter of the second right leg; but for its small size the supernumerary leg presents a normal appearance.

The hosts have been recorded for all of the specimens described in 1909 and for 8 out of 11 specimens recorded in this paper. It is therefore clear that abnormal ticks, such as are here described, are not incapacitated from acting as parasites.

It appears worthy of note that in 7 out of 10 specimens (including the two figured in 1909) showing underdevelopment of one side of the body it is the right side which is underdeveloped—this perhaps may not be a mere coincidence. It is only in specimens Nos. 2 and 9 and partially in No. 6 that the left side is underdeveloped. In specimen No. 7 the festoons of both sides are abnormally formed.

PENETRATION OF *IXODES* BENEATH THE SKIN.

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

In *Ticks* (Part II. p. 314) various cases are cited by me in which *Ixodes* in the larval, nymphal and adult stages have been found lodged beneath the skin of the host: Dubreuilh (1838), Van Beneden (1883) and Blanchard (1891) record cases in man where ticks were found either in a pustule or in cysts beneath the skin. In Blanchard's case a living *I. ricinus* (♀), 8 mm. in length, was excised from the patient, in whom it caused a subcutaneous tumour about the size of a nut which had appeared some weeks before. Mégnin (1867 and 1892) found (?) *ricinus* nymphs beneath the scabs covering pustules on the legs of a horse at Versailles. Kossel, Schütz, Weber and Miessner (1903) state that *ricinus* nymphs and larvae occasionally bore themselves beneath the skin of cattle.

Trilebert (1863) observed a cyst containing (?) *ricinus* at the end of a dog's ear, and Aurivillius (1886) records the presence of *ricinus* beneath the skin of a fox.

In March of this year, Mr Charles L. Walton, of the Zoology Department, University College of Wales, sent us some specimens (N. 2733–2735) obtained from beneath the skin of two foxes by Mr Hutchings, the local taxidermist at Aberystwyth. Some four or five ticks occupied as many "cysts" beneath the ear and about the groin of the animals. On examining two of the ticks (the remaining cysts were preserved unopened) we found them to be *I. ricinus* (♀) and *I. hexagonus* (nymph). This appears to be the first case of the kind recorded in Great Britain. The cysts were preserved in alcohol and tightly enclosed the ticks, which were removed with difficulty from the tough connective tissue in which they were embedded.

The mechanism whereby *Ixodes* may occasionally penetrate beneath the skin appears to me easy of explanation. It is an unusual and abnormal process and I believe, in the absence of any other explanation, that it depends upon the amount of oedematous swelling of the host's skin about the site of attachment of the tick. When the mouthparts of the latter become implanted in the firm subcutaneous connective tissue and the reaction about the puncture leads to oedema, it follows that the oedematous tissue must rise and gradually envelop the tick to a greater or less extent. In some large ticks, like *Amblyomma hebraeum* and *A. variegatum*, the ticks are frequently found partly embedded with their capitulum and part of their body inserted into the crater of what may be described as a miniature volcano or cone of oedematous skin. In *Amblyomma* the large size of the tick appears to preclude their complete disappearance beneath the skin, whereas in *Ixodes* the ticks' bodies, being smaller, penetration will be more readily effected. To repeat, the penetration of *Ixodes* beneath the skin is not due to any mechanical activity on the part of the tick ; it is due to the increasing oedema and inflammatory swelling of the host's skin whose surface rises above the subjacent tissues in which the tick's hypostome is anchored—whilst the tick is sucking the host's blood the host's skin revenges itself by swallowing the tick. After the tick has penetrated beneath the skin, the wound it produced may heal and be obliterated. The long hypostome in *Ixodes* appears to be an essential factor in the process, for we have no records of Ixodidae with short hypostomes penetrating beneath the skin. However long the ticks may live in this situation, the firm cyst-like mass of tissue which forms about them necessarily renders their subsequent escape impossible and they must die *in situ*.

NOTE ON A LEG ABNORMALITY IN *ACANTHIA LECTULARIA.*

By EDWARD HINDLE, B.A., Ph.D.

(*From the Quick Laboratory, Cambridge.*)

(With 1 Text-figure.)

Whilst engaged in rearing a number of bed-bugs, the variation recorded below was observed in an individual that had just moulted from the first to the second nymphal stage. As such an abnormality does not appear to have been previously described, it seemed worth while to place it on record.

The accompanying figure (Fig. 1) shows that this individual possesses a striking peculiarity in the conformation of its three right legs. On the left side the legs are normally developed and as in most insects are divided into the following segments commencing with the proximal one: coxa, trochanter, femur, tibia, and tarsus. In the adult bug the tarsus is three-jointed, but in the early nymphal stages it is impossible to detect any signs of these segments.

In this example, on the left side the legs are not more than half the size of those on the right and in addition the segmentation is irregular.

In the first leg the femur and tibia seem to be fused together and from the middle of their length arises a bifid protuberance covered with bristles at its two distal extremities. The remaining segments of the limb are of normal aspect but, with the possible exception of the coxa, are incompletely developed.

The second right leg resembles the first, there being no distinct articulation between the femur and tibia, whilst a bifid protuberance arises from what may be regarded as the junction of the two segments. In both cases this protuberance seems to be articulated with the fused femur and tibia, and to constitute a distinct, though incompletely developed, appendage.

In the third right leg the coxa is normal, and the trochanter, femur and tarsus, although miniature, resemble those of the corresponding

Fig. 1. *Acanthia lectularia.* Ventral view of abnormal second stage nymph (× 60).

leg on the opposite side, but the tibia is divided into two segments by an articulation in the middle of its length. There is no trace of the rudimentary appendage present in the first two legs.

ON THE ANATOMY OF A FOWL TAPEWORM, *AMOEBOTAENIA SPHENOIDES* VON LINSTOW.

By F. J. MEGGITT, M.Sc.

(*Board of Agriculture and Fisheries Research Scholar, University of Birmingham.*)

(With Plates XX and XXI.)

CONTENTS.

Introduction.

In the course of an investigation into the tapeworms of poultry, I came across a minute form (*Amoebotaenia sphenoides* von Linstow) hitherto unrecorded from this country, and incompletely described from the few specimens examined by other workers. Its economic importance remains to be ascertained but, pending a complete experimental treatment of its life-history, I submit an account of its anatomy.

The specimens were obtained from fowls in the neighbourhood of Harborne, Birmingham. Every one of the fowls dissected was infected with this parasite, as many as 40 to 50 specimens being found in one bird.

It is curious to note that, as far as could be ascertained, none of the characteristic symptoms of tapeworm disease were present, *i.e.* the birds were not specially thirsty or listless, the appetite was normal, blood and mucous were absent from the faeces, and there were no signs of intestinal catarrh : except for a rather pronounced thinness upon the breast, the birds were normal in every way. There has also been an entire absence of symptoms in birds which I have afterwards found to be infected with *Davainea proglottina.* It is possible, therefore, that the rather vague symptoms given by various authors (Stiles) were based upon extreme cases, and that in the normal course of events, especially with the smaller species of Cestodes, no such symptoms are to be expected.

On the other hand, infection with common Nematodes such as *Trichosoma collare, Heterakis vesiculare*, and especially *H. inflexa*, produces pronounced symptoms agreeing in all respects with those attributed to Cestodes.

A. sphenoides, so far as I am aware, has hitherto not been recorded from Great Britain. It has been found in Germany (von Linstow), Lombardy (Grassi and Rovelli), Brazil (Magelhães), and Maryland and Columbia, U.S.A. (Ransom). It is probably more common than is suspected : several times in dissecting fowls sent for examination, there have been found isolated proglottides resembling those of *A. sphenoides.* On account of its small size, the head, when detached from the strobilus, is very difficult to see and thus, in the cases just referred to, has probably escaped detection. A more accurate examination of the intestines of ordinary fowls would, in all probability, show that these parasites, and tapeworms in general, are widely distributed throughout Great Britain.

The work has been carried out in the Research Laboratory in Agricultural Zoology of the University of Birmingham, under the supervision of Prof. F. W. Gamble, F.R.S., to whom I am deeply indebted for many valuable suggestions and criticisms.

Historical.

The original description of this species was given by von Linstow, based chiefly upon external characters. It was next recorded by Piana from Italy, and later on by Magelhães from Brazil : both these investigators, however, confined themselves to external characters and the more striking internal organs. Cohn (1901) was the first to give a true description of the internal anatomy, a description accurate as far as it went, but still leaving many points to be elucidated.

The nomenclature has undergone many changes. The species was first called *Taenia cuneata* by von Linstow. Railliet altered it in successive years to *Taenia sphenoides*, *Dicranotaenia cuneata*, and *D. sphenoides*. In 1899 Cohn created a new genus, *Amoebotaenia*, for it, making it the type species and calling it *A. sphenoides*, a name which he afterwards changed to *A. cuneata*. Under this name it was catalogued by Fuhrmann ; Ransom finally altered the name to *A. sphenoides*, the specific name "*cuneata*" having already been preoccupied.

In common with most bird Cestodes, nothing is certainly known of the life-history. Grassi and Rovelli (1892) found in the brandling (*Allolobophora foetida* Eisen) cysticercoids which they believed to be the young stage of *A. sphenoides*, an assumption based upon comparisons of the hooks of the two forms, and upon the fact that the cysticercoids could not possibly be the larval stage of any of the other tapeworms found in the vicinity. No feeding experiments were made however, so that the assumption still remains unproved.

External Characters.

The wedge-shaped form described by various investigators is very characteristic of this species, and affords a convenient method of identifying it roughly from other tapeworms. This only applies to young and mature forms however : as the cestode becomes older, the more posterior proglottides cease to increase more rapidly than the preceding ones, so that the greatest diameter of the strobilus is at about the posterior quarter of the worm ; after that the worm begins to taper off again (Pl. XX, fig. 1). The number of proglottides varies according to the age and completeness of the specimen. Magelhães and von Linstow found 13 the maximum, while Cohn's specimens had 19 and 24 : I have often had one with 20 and once one with 23.

In shape each proglottis is like a very much truncated pyramid with an elongated rectangular base, the smaller end being directed anteriorly. Seen horizontally, it is much wider than long, and both anterior and posterior margins are slightly curved posteriorly. A longitudinal vertical section is rectangular with a small semicircular projection anteriorly and a corresponding semicircular depression posteriorly (Pl. XXI, fig. 10). The groove this depression causes runs from side to side of the proglottis, but gradually vanishes towards each lateral margin so that the extreme end sections of a proglottis show no signs of it. The rounded anterior extremity of each proglottis fits into the

groove in the posterior extremity of the preceding one, the proglottides thus fitting telescopically into one another. Cuticle and sub-cuticula are absent from the groove and from the anterior margin, so that the parenchyma of one segment is in direct communication with that of another. This can easily be seen in mature proglottides, when the ripe eggs from one may intrude into a neighbouring one: as a rule, in the posterior part of the strobilus, there is no internal separation whatever. A younger detached proglottis shows anteriorly and posteriorly a distinct parenchymatous boundary.

The following table gives the external measurements according to various workers. It can be easily seen that there are discrepancies, though these might be expected. The active movements of the worm so alter its size and shape that it is impossible to measure accurately a live specimen: measurements thus obtained may double or halve themselves according to the state of expansion or contraction at the time. In a similar manner, measurements, especially of the head, of dead specimens are unreliable owing to the varying shapes assumed by the worm when dying, and the consequent apparent alteration in size. The figures given below are only approximately correct, those occurring most frequently in a large number of measurements having been taken as normal. It is apparent that sizes exceeding these have been obtained, and that these extremes cover and thus neutralise the discrepancies before mentioned.

	Length	Length of head	Breadth of head	Thickness of head	Length of rostellum	Breadth of rostellum	Length of hooks
von Linstow	2	—	—	—	—	—	0·032
Magelhães ..	3–4	0·2	0·205	—	0·15	0·0375	0·025
Cohn ..	2·75	0·14	0·2	—	0·15	0·037	0·025
Meggitt ..	2·5	0·213	0·162 0·220	0·098	0·167	0·039	0·026

	Size of suckers	Size of egg
von Linstow	—	—
Magelhães	0·075	0·042
Cohn	0·11	0·028
Meggitt	0·1218 0·0784	0·0294

All measurements in mm.

Rostellum.

The head is provided with four suckers and a well-developed apical rostellum.

The rostellum (Pl. XX, figs. 5 and 6) consists of two parts, a muscular protrusable axis (*a*), and an ensheathing case (*c.r.*). The latter is

elliptical in longitudinal section with the greatest diameter at approximately the anterior third of its length. At its apex, the anterior end has a narrow circular opening (*o*), leading by a channel of the same width into a dome-shaped chamber (*d.c.*) through the floor of which protrudes the muscular axis (*a*). The sheath (*c.r.*) consists of a dark staining structureless membrane, continuous with the body cuticle, inside which is a parenchyma similar to, but more compact than, the parenchyma of the body.

The muscular axis is a narrow cylindrical organ, having anteriorly a terminal portion much wider than the remainder of the axis, from which it is separated by a deep constriction. This terminal portion is armed with a circle of hooks of the form characteristic of this genus, arranged with the heads nearly meeting anteriorly at its apex, and the hooks directed posteriorly, parallel to and in the same plane as the longitudinal axis of the rostellum. Posterior to the head, the remainder of the axis, after a slight bulge, tapers to a rounded extremity, the extreme end in the contracted condition being bent round upon itself so as to be directed anteriorly. The axis itself is formed of elongated muscles with distinct nuclei: its outer margin is formed by a dark staining membrane like that of the sheath, surrounding a layer of circular muscles (*c.m.*): the enlarged terminal portion in addition has an external layer of extremely thick and dense cuticle (*c.h.*) in appearance much like the substance of the hooks. Thick muscles (*r.m.*) extend from the sheath into the surrounding parenchyma and serve for the partial retraction of the sheath, the anterior end of which usually protrudes a little beyond the scolex (Pl. XX, fig. 7).

In the contracted condition, the enlarged terminal portion of the axis occupies and just fills the dome-shaped chamber in the sheath (Pl. XX, fig. 5): when protruded, it projects from the anterior opening of the sheath, the lips forming the walls of the sheath being forced apart to allow of the passage of the body of the axis. In the extruded condition it can be seen that the walls of the dome-shaped chamber have only been pushed aside, not protruded: it follows, therefore, that the axis has simply elongated itself through the floor of the chamber and consequently is unattached to it, merely filling the opening. There are apparently no muscles connected with the sheath, except those for its retraction, so that the muscles necessary for the protrusion of the axis must be contained within itself. The contraction of the circular muscles at the same time as the elongation of the longitudinal ones would cause an elongation of the axis as a whole, the extent of the elongation being

sufficient to protrude the head through the anterior opening of the sheath.

Cuticle and Musculature.

Owing to the extremely small size of this species, it would be unprofitable to make any detailed observations upon the nicer points of the anatomy owing to the impossibility of sufficient accuracy. The following section consequently deals only with the more prominent points.

There is a distinct cuticle, finely granular in some sections, in others apparently homogeneous: this contradiction is probably due to the varying action of the fixative upon a tissue in process of being sloughed off. The cuticle is bounded internally by a thin basement membrane, and externally has a slight roughening of the surface due apparently to a multitude of extremely fine and densely packed rods. This can be seen however only in specimens treated with special care, the external layer seeming to be brushed off by the lightest touch.

Under the cuticle is a single layer of longitudinal cuticular muscle fibres: in transverse sections, these appear only as extremely small dots in a granular matrix. Of circular cuticular muscles no trace can be seen, unless the granular matrix just mentioned represents them.

A definite sub-cuticular lies under the cuticular muscles. It consists of elongated, roughly columnar, cells lying at right angles to the surface of the body, each with a darkly staining nucleus and granular contents. Tangential sections show that their outer faces are polygonal, fitted together to form a mosaic: further inwards they separate, leaving spaces between them, so that their inner ends do not touch one another. Scattered through this sub-cuticular are spherical spaces which possibly represent spaces filled in life by calcareous bodies. None have been recorded from this species however, and I have been unable to determine definitely their presence in fresh or preserved material.

Under the sub-cuticular is a well-developed system of longitudinal muscles. These run as isolated bundles from the scolex to the end of the proglottis, each bundle consisting of several fibres. Their course is not exactly straight owing to the form of the proglottides. At the junction of each proglottis with its neighbour they bend inwards, sharply bending out again as soon as they reach the next proglottis.

Excretory System.

The excretory system (Pl. XXI, fig. 9) consists as usual of four longitudinal vessels, one dorsal (*d.l.c.*) and one ventral (*d′.l′.c′.*) each side, running the whole length of the strobilus. Of these four vessels, the two ventral are much smaller than the two dorsal and at times become so small that they appear to vanish. The course of each longitudinal vessel is not straight. In each proglottis it has the form of an arc with the convex side directed towards the exterior: the arc extends externally approximately to the level of the inner end of the cirrus sac; it passes from one proglottis to another about at one-third of the width of the strobilus and has there its innermost margin. The four canals are joined at the end of each segment by an elliptical commissure: the transverse commissures (*t.c.*) between the two dorsal and the two ventral vessels are approximately the width of the respective longitudinal vessels and are therefore fairly obvious: those between the two vessels of the same side are very minute (*v.c.*).

The excretory system in the scolex is much more complex, although it still conforms to a definite plan. There is no neck, so that the longitudinal vessels pass straight into the head system. At the level of the posterior margin of the suckers each longitudinal vessel curves slightly towards the interior and then runs in a straight line along the rostellum to the level of the anterior border of the sucker. Half-way along its course each sends off a wide branch to the rostellum (*r.c.*): and at each third of the course the two vessels of each side communicate with one another by small transverse commissures. Arrived at the anterior margin of the sucker, each describes a semicircle under the cuticle of the scolex: the two semicircles of each side converge as they approach the exterior and finally join on the lateral margin of the strobilus, half-way between the suckers. The common vessel thus formed only extends for a short distance, splitting again into two, each of which (*lat.c.*) runs posteriorly, equidistant from and in the same horizontal plane as its corresponding longitudinal vessel. Arrived at the posterior margin of the sucker, each lateral vessel describes another semicircle, opening into its corresponding longitudinal vessel. Two loops are thus formed on each side, communicating with one another at their extreme antero-lateral limits. The two lateral vessels on each side communicate with each other by small transverse commissures and at about the posterior third of its course each gives off a small vessel running to the corresponding sucker (*s.c.*). The corresponding lateral and longitudinal vessels

also communicate with each other once or twice. Apart from the commissures in each proglottis, the vessels on opposite sides communicate with one another in the scolex by two ring commissures, one nearly at the posterior limit of the rostellum (*p.r.c.*) just where the lateral vessels join the main longitudinal one, and one at the extreme apex of the rostellum (*a.r.c.*). At the point where each main longitudinal vessel bends round to form the lateral vessel, a narrow branch (*x*) is given off anteriorly. This runs in a straight line, continuing the course of the longitudinal vessel, to the extreme apex of the rostellum : there it opens into the circular commissure (*a.r.c.*) running exactly under the cuticle and joining the four branches. In addition to this main plan there are numerous fine branches which run to the suckers and rostellum and anastomose with one another to such an extent that, owing to their small size, it is impossible to trace their course with any degree of certainty.

Nervous System.

The nervous system of this species (Pl. XXI, fig. 11) has been entirely neglected by previous investigators. It consists essentially of two longitudinal nerve trunks (*l.n.*) joined in the scolex by two commissures, one dorsal and one ventral. Each nerve trunk extends from the posterior margin of the strobilus to a little below the centre of the suckers : it runs as usual in the parenchyma, externally to the excretory system, but internally to the musculature. At about the level of the posterior third of the suckers, the nerve is joined to its fellow of the other side by two commissures. These commissures do not form a nerve ring proper, but run postero-laterally round the rostellum so that their dorsal and ventral surfaces are considerably posterior to their junction with the nerve trunks. Each commissure is much swollen on the ventral and dorsal surface of the rostellum to form a small crescent-shaped " brain " (*b*) : the two junctions with the nerve trunks are also much swollen to form two lateral ganglia (*l.g.*) surrounded with dense masses of deeply staining cells. The whole circum-rostellar ring thus encircles the posterior third of the rostellum. On each side of the lateral ganglion, a small nerve is given off : this runs outwards and slightly anteriorly to the centre of the corresponding sucker (*s.n.*) ; each sucker being supplied by one of the four nerves from the circum-rostellar ring. Slightly dorsal and ventral to the sucker nerves two other nerves are given off (*r.n.*), one on each side of each of the lateral ganglion as before. These nerves run anteriorly nearly to the apex of the rostellum where each branches

into two at right angles to its former course, one branch running to the apex of the rostellum ($r'.n'.$), and one down the margin of the scolex ($s'.n'.$) apparently to the anterior margin of the corresponding sucker. Each sucker would thus appear to be supplied by two nerves. The main rostellar nerve is in the form of an arc with the convexity directed towards the suckers. A thick branch is given off to the rostellum soon after it leaves the circum-rostellar commissure.

Genital Organs.

Each proglottis has a common genital sinus, regularly alternating, situated at the anterior sixth of its length, almost at its anterior corner (Pl. XX, fig. 4). It is cylindrical in shape with the inner end rounded. It has a breadth of 0·0127 mm., and extends 0·04 mm. into the proglottis, making an angle of 40° with the side of the worm. Into it open the cirrus and the vagina, not side by side as Magelhães states, but one in front of the other, the vagina opening into the extreme end of the sinus, and the cirrus into the side some little distance from the vagina. My specimens thus confirm Cohn's description but not his figure: the latter indeed does not agree with his own statements, for he does not figure a genital sinus at all. The sharp anterior bend of the vagina after leaving the genital pore gives at first the impression that the sinus is situated much more posteriorly, and to this appearance is probably due Cohn's statement that the pore is at the anterior third of the proglottis.

Male Organs.

According to Magelhães, "Le parenchyme des deux premiers anneaux ne présente aucune trace d'organes génitaux. Les organes mâles se montrent déjà dans le troisième anneau." On the other hand Cohn states, "Die ersten Zellhaufen, welcher die später zur Entwicklung gelangenden Genitalorgane bezeichnen, sind schon früher vorhanden, als sich selbst die erste Proglottis deutlich absondert. Zwei solche Zellhaufen, durch ihre Färbung im Totalpräparat deutlich kenntlich, liegen noch im ungegliederten Theil gleich hinter den Saugnäpfen und in der Mittellinie." My own preparations agree with neither statement. The first signs of male organs usually appear in the second segment, although I have seen dubious traces in the first. Of Cohn's "Zellhaufen" behind the suckers I have been unable to find any trace. On the whole, the male organisms are first clearly recognisable as such in the third segment. It is possible that with the increasing age of the worm, the earlier

segments ripen sooner : this would account for the discrepancies in the statements of Cohn and Magelhães.

The testes are spherical bodies, 12 in number and 0·045 mm. in diameter, lying in a single row on the dorsal surface of the posterior end of the proglottis (Pl. XX, fig. 4). From the end testis on each side, a narrow vas deferens runs along the posterior margin to the centre, receiving on the way a number of short and minute vasa efferentia from the testes, one vas efferens from each testis. Arriving at the centre of the proglottis, the two vasa deferentia unite to form a single duct running anteriorly to the anterior border of the proglottis. There it bends at right angles to its previous course and forms a series of elongated coils, after which it enters the cirrus sac. As Cohn states, there is no vesicular seminalis, the coils of the vas deferens being greatly dilated and filled with spermatozoa, thus functioning as one. The walls of the coiled portion of the vas deferens consist of a slight muscular layer and an outer membrane, external to which is an irregular layer of nuclei. On the smaller ducts no observations could be made owing to their small size.

The cirrus varies greatly in position in the different segments : it often extends horizontally into the proglottis but may be so directed that its hinder end touches the posterior border of the proglottis and its front end the anterior border : there are all stages between these two extremes. All cases though agree in having a sudden twist, either posteriorly or anteriorly, in the anterior quarter of the sac.

The vas deferens (0·0046 mm. diam.) enters the cirrus sac at the posterior end and runs with one or two curves as far as the bend just mentioned. Here it doubles back upon itself, extending nearly to the posterior end, when it again bends round to open into the cirrus. This latter is almost straight and runs through the anterior half of the cirrus sac and the bend to open into the sinus close to the opening of the vagina.

The cirrus sac consists of a definite dark staining membrane enclosing a space almost entirely filled by the vas deferens and cirrus, the interstices between them being occupied by a fibrous tissue. Numbers of large nuclei are scattered through the sac. The vas deferens itself, owing to the increased width of the muscular layer, is slightly wider inside the sac than outside, and has a peculiar circular striation at right angles to its length : otherwise it has the same structure as before. I should assume this striation to be due to the presence of circular muscles, although the rings are relatively so far apart that they appear entirely isolated from one another. The cirrus itself is a

straight narrow tube with a diameter of 0·002 mm. It has a thin wall which stains intensely black with haematoxylin and is probably of a chitinous nature.

Female Organs.

With regard to the appearance of the female genital organs, Cohn states, "In die sechsten Proglottis sind neben den gut entwickelten, wenn auch noch nicht reifen Hoden das Ovarium und der Dotterstock nur eben angelegt." My own specimens do not confirm this statement. I have found unmistakable traces of a vagina and yolk-gland as early as the fourth segment, and possibly more careful staining would reveal them in preceding proglottides, Magelhães having found the vagina in the third. Neither can the following statement be regarded as correct : "Nachdem sie (*i.e.* the female genital organs) aber einmal im 11–12ten Gliede das Maximum der Entwicklung erreicht haben, verschwinden sie mit einem Schlage, so das im 13ten Gliede, das bereits ganz mit dem Uterus gefüllt ist von Ovarium und Dotterstock keine Spur mehr vorhanden ist."

The female genital organs persist as far as the 14th proglottis in which yolk-gland, ovary, and receptaculum seminis can be seen quite distinctly. Posterior to this they disappear gradually, the yolk-gland being quite distinct in the 16th segment. The uterus which fills the 13th segment according to Cohn is probably the ovary, which is there fully developed and filled with eggs so as to resemble a mature uterus : the uterus really is not properly developed until the 16th segment but remains overlaid by the ovary.

The ovary (Pl. XX, fig. 4, *ov.*) lies on the ventral side of the proglottis, a little to one side of the median line, and half-way between the anterior and posterior borders. It consists, at first, of two short wings connected by a long narrow isthmus, the wing nearest the genital pore being slightly the smaller. In the more posterior segments however, it increases considerably in width, becoming less rope-like, and curls considerably ; the isthmus often touches the anterior border of the proglottis while the ends reach the posterior border. It is while the ovary is in this condition that maturation of the eggs occurs. In the following segments the ovary quickly disappears, no trace of it being found in the 16th.

The yolk-gland (*y.g.*) also lies on the ventral side of the proglottis, and directly posterior to the isthmus of the ovary. It is a flat compact disc-shaped organ composed of a number of roughly spherical cells with

darkly staining nuclei and has a diameter of 0·0512 mm. It first appears about the 6th segment, becomes functional in and persists as a vestiger until the 16th.

The shell-gland (Pl. XX, fig. 2) surrounds the vestibule as it passes directly dorsal to the yolk-gland. Its edges touch and appear to fuse with those of the yolk-gland: this causes it to appear merely an irregular proliferation of the latter: it was probably this appearance which caused Cohn to overlook its occurrence. The two glands are quite distinct however in character, for while the yolk-gland is composed of spherical cells the cells of the shell-gland are irregular in shape and have a more irregular distribution of the nuclei: the gland itself lacks the compactness of the yolk-gland.

The vagina (Pl. XX, fig. 4) opens into the extreme end of the genital sinus, posteriorly to the cirrus, the opening having a diameter of 0·0062 mm., and being surrounded by the usual well-developed sphincter muscle. The vagina immediately bends sharply anteriorly and then runs horizontally past the end of the cirrus sac, nearly to the dorsal margin of the proglottis. Bending ventrally again, it opens through a constriction into the broad end of an elongated pear-shaped receptaculum seminis, situated in the centre of the segment. The receptaculum seminis is 0·093 mm. long and 0·0391 mm. wide at its broader end, 0·0207 mm. at the other. This latter end (Pl. XX, fig. 2) curves ventrally and then divides into two. The broader branch (*ov.d.*) continues to curve ventrally as far as the ventral border of the proglottis: there it runs anteriorly, and slightly towards the cirrus-sac side of the segment, to open into the ovary near the anterior margin of the proglottis.

The narrower branch or vestibule (*ves.*) runs at first anteriorly, but almost immediately doubles back upon itself and runs posteriorly and dorsally to pass through the shell-gland near the posterior margin. The vitelline duct here opens into it on the ventral surface. This latter is a short duct posterior to the shell-gland, joining the vestibule and the yolk-gland. After passing through the shell-gland, the vestibule curves ventrally and then runs antero-laterally, still on the dorsal side of the receptaculum seminis, to the centre of the anterior margin of the proglottis. It then bends ventrally at right angles to its former course, and expands in the middle line into the uterus (*u.*).

My own specimens confirm Cohn's opinion that this is from the beginning a simple sac: there seems to be no grounds for Magelhães' statement, " Les œufs se développent à l'intérieur de 13 à 15 grosses vésicules ou cavités rangées suivant le bord postérieur de l'anneau

adulte. Quand ils sont mûrs, ils ont fait éclater les vésicules en question et sont disséminés dans toute l'entendue de l'anneau : cela s'observe notamment à partir du douzième anneau et sur les anneaux isolés par rupture spontanée." Of the "13 à 15 grosses vésicules ou cavités" in question I can find no trace : the persistent testes are the only organs present answering to this description.

The uterus makes its first appearance in the 12th segment as a small bag against the anterior border, slightly to one side of the middle line. It rapidly develops, displacing the ovary in the following segment and masking it in the 14th. By approximately the 16th segment it extends as far as the lateral border of the proglottis, overlapping the excretory canals : previous to this, the lateral quarter of each proglottis consists of parenchyma. Here the uterus changes its form ; previously it has the form of an elongated sac ; it now sends out finger-like outgrowths posteriorly (Pl. XX, fig. 8), which in the following segments increase in size, reaching the posterior border of the proglottis. The eggs are closely packed together in the uterus so that their shape owing to compression is generally polygonal. In the 19th segment the outer growths have apparently anastomosed with one another so that the whole proglottis is now merely a huge sac filled with eggs, and for the first time these conform to the characteristic Cestode type of an onchosphere within a thin and translucent envelope.

The mature proglottis is merely a pouch of eggs when detached from the strobilus. In sections it may be seen that the eggs are not enclosed in any definite wall, but are in a large bunch occupying the centre of the segment and surrounded by a ragged irregular parenchymatous border. The uterus at its first commencement is a definite organ with well-defined walls. It is evident, therefore, that somewhere between the 12th and 19th segments a change must have occurred, the uterine wall must have burst and the eggs have invaded the parenchyma, pushing back the latter as they increase in size until it became a mere shell enclosing the eggs. This change occurs in the 19th segment. Previous to that the eggs are polygonal, showing compression, the uterus has a definite shape and does not fill the whole proglottis : subsequent to and including the 19th proglottis, the eggs become spherical and are scattered through the proglottis, and the uterus apparently loses its shape. In one whole mount, by a lucky accident, I could actually see the wall in the 18th segment ; while in the 19th it was obvious that no wall could be present. The whole change is probably due to the pressure caused by the growth of the eggs.

Life-history.

The only work in connection with the life-history has been done by Grassi and Rovelli (1892) who found this form in Lombardy. From pens inhabited by infected fowls they obtained many brandlings (*Allolobophora foetida* Eis.) containing cysticercoids. After a careful examination of these cysticercoids and a comparison with adult forms found in the various vertebrates in the vicinity, they came to the conclusion that these must be the larval stages of *Amoebotaenia sphenoides*. No feeding experiments were made however, so that the life-history still remains hypothetical.

My own investigations have so far only yielded negative results. In the two fields to which the infected poultry have access, I have never found any trace of brandlings whatever. This result seems to point to the conclusion that, in England at least, the intermediate host is another species. This cannot be said to be altogether unexpected, for it may be recalled that of the three species of slugs (*Limax cinereus*, *L. agrestis*, and *L. variegatus*) recorded by these investigators as intermediate hosts of *Davainea proglottina*, one the more usual (*L. variegatus*) is not a British form. It is therefore quite possible that the Cestode in question, *Amoebotaenia sphenoides*, may have different hosts in England, Europe and South America.

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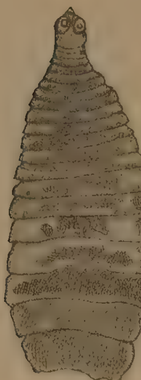
DESCRIPTION OF PLATES XX AND XXI.

The following letters apply to all the figures

a.	Axis of rostellum.
a.r.c.	Anterior ring commissure.
b.	"Brain."
c.	Cirrus.
c.h.	Cuticle of head of rostellum.
c.m.	Circular muscles.
c.r.	Rostellar sheath.
c.s.	Cirrus sac.
d.l.c.	Dorsal longitudinal canal.
d′.l′.c′.	Ventral longitudinal canal.
d.c.	Dome-shaped chamber.
g.s	Genital sinus.
l.c.	Longitudinal excretory canal.
l.g.	Lateral ganglion.
lat c.	Lateral canal.
l n.	Main longitudinal nerve.
o.	Opening of dome-shaped chamber.
ov.	Ovary.
ov.d.	Oviduct.
p.r.c.	Posterior ring commissure.
r.	Rostellum.
r.c.	Rostellar canal.
r.m.	Retractor muscles.
r.n.	Main rostellar nerve.
r′.n′.	Rostellar nerve.
r.s.	Receptaculum seminis.
s.c.	Canal to sucker.
s.n.	Nerve to sucker.
s′.n′.	Supposed nerve to sucker.
sh.g.	Shell-gland.
t.	Testis.
t.c.	Transverse excretory canal.
u.	Uterus.
v.	Vagina.



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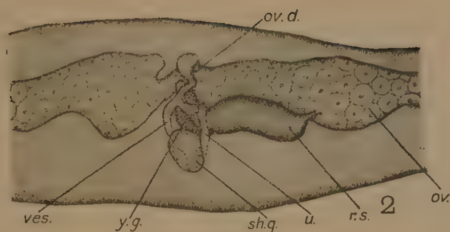
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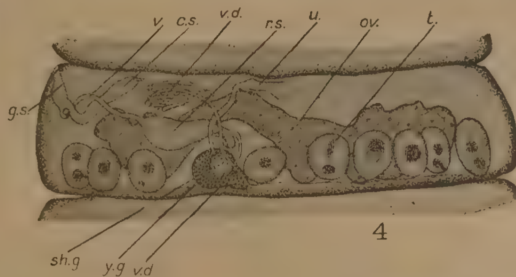
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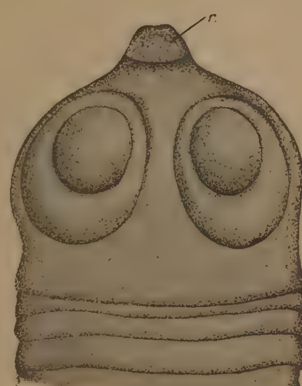
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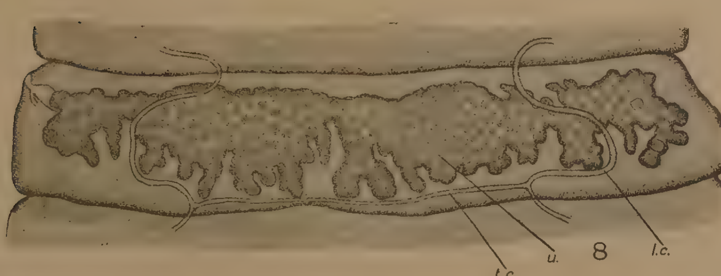
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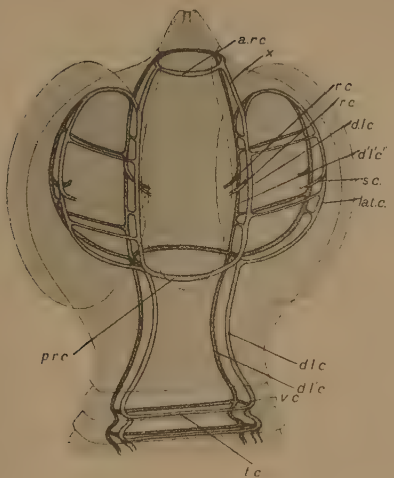
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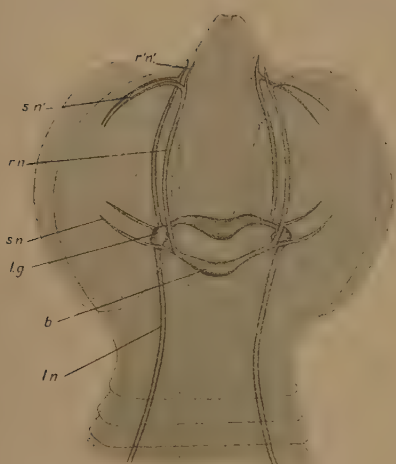
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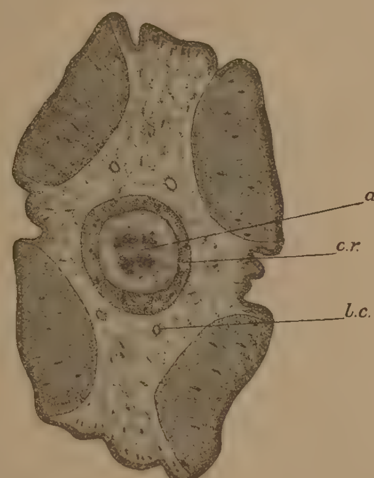
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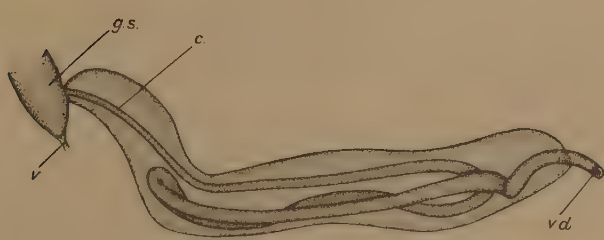

g.s.
c.
v
vd

13

ves.	Vestibule.
v.c.	Canal joining dorsal and ventral vessels.
v.d.	Vas deferens.
y.g.	Yolk-gland.

PLATE XX.

Fig. 1. The whole strobilus, stained. ×22.
Fig. 2. Reconstruction from sections of the female genital ducts.
Fig. 3. Mature egg, stained. ×374.
Fig. 4. Dorsal view of a stained preparation of a proglottis with mature sexual organs. ×150.
Fig. 5. Rostellum retracted. ×185.
Fig. 6. Rostellum protruded. ×185.
Fig. 7. Scolex. ×189.
Fig. 8. Stained preparation of a proglottis, nearly ready for detachment. ×120.

PLATE XXI.

Fig. 9. Freehand reconstruction of the excretory system. ×77.
Fig. 10. Longitudinal vertical section through a proglottis. ×565.
Fig. 11. Freehand reconstruction of the nervous system. ×77.
Fig. 12. Transverse section of the head through the rostellum and suckers. ×352.
Fig. 13. Cirrus sac of *Amoebotaenia sphenoides*. ×750.

NOTES ON THE ANATOMY OF THE BED BUG, (*ACANTHIA*) *LECTULARIA* L.[1]

(With special reference to uncorrected errors of previous investigators.)

By C. HAY MURRAY, D.Sc.
The Free Public Museums, Liverpool.

(With Plates XXII and XXIII and 40 Text-figures.)

Introduction.

In these days of close research into the activities and the anatomy of various insects, it is surprising that the structure of the common, indeed almost ubiquitous, Bed Bug, *Acanthia* (*Cimex*) *lectularia*, has so long escaped the thorough investigation which is called for by the standards of modern science.

Dr Leonard Landois, Assistant in the Anatomy and Physiology Institute of the University of Greifswald, has long held the field as the "authority" on the subject. As, however, his treatise *Anatomie der Bettwanze* was published so far back as 1868–69, it was more than likely that the inevitable inferiority of his appliances to those now at the command of the investigator, must have landed him in errors which could now be avoided. The writer therefore decided to do the whole research work *de novo*, without reference to what had previously been done by others, so that his conclusions would be based on personal observation with no bias from suggestion. This being done, he succeeded, after much trouble, in obtaining Landois' publication (1st half) in the original German, which does not seem to have been translated into English, and found that his expectation had been correct. Owing, probably in some degree to the lack of a microtome,

[1] The more outstanding discrepancies between Landois' work and that of the writer were laid before the Association of Economic Biologists, whose meetings were held in the University of Liverpool on December 31st, 1913. Prof. R. Newstead read the paper on behalf of the writer.

the work, although painstaking and full of acute observation, was in many places defective and inaccurate. The writer therefore submits the results of his own original research, indicating where and how his findings differ from those of Landois, having in every case of difference done that part of the work over again, with, if possible, still greater care, so that he might the more confidently affirm the truth of his contentions.

The following is the general description of the bed bug given by Messrs Douglas and Scott in their work *The British Hemiptera*, vol. I.

Acanthia Fabricius.

"Flat, broad, ovate.

Head: broad, convex; sides behind eyes straight.

Face is long, broad, convex, triangular, the base of the triangle being in front projecting between the antennae; antenniferous processes very stout and much deflected. Underside with a rostral channel.

Antennae: 1st joint stout, not reaching to the end of the face; 2nd joint four times as long but not so thick as the 1st, and very slightly thickened to the apex; 3rd and 4th joints very thin filiform; 3rd as long as 2nd; 4th two-thirds as long as 3rd.

Eyes: inserted at the front of the sides of the head, large, somewhat flattened, prominent.

Rostrum: the joints in length subequal; 1st at the base enclosed by the labium and cheek plates; 2nd broadest; 3rd thinnest, apex acute.

Thorax: Pronotum subreniform; much wider than long, anterior margin very concave, not raised into an annulus; side margins foliaceous more or less reflexed, rounded in front, projecting; exterior angles rounded; sides gradually narrower to the hinder angles which are obtuse, almost right angled; posterior margin straight; disc concave posteriorly depressed with a strong ridge within the hinder angles.

Scutellum: broad, short, triangular, rather convex.

Elytra: without clavus, cuneus, or membrane. Corium as wide but scarcely so long as the first segment of the abdomen; posterior margin slightly rounded. Wings wanting or rudimentary.

Sternum: mesosternum in the centre between the coxae with a long flat trapeziform plate which hindwardly projects over the metasternum.

Legs: strong; coxae 1st pair close together; 2nd and 3rd pairs laterally distant. Thighs all subclavate, thickest at the base.

Tibiae of equal size throughout.

Tarsi 1st joint short; 3rd longer than 1st and 2nd together; claws long.

Abdomen: oval, flat, base much wider than pronotum, connexivum very narrow. Greatest breadth rather behind middle. Underside on the second segment in the middle a long thick trapeziform flat plate, directed forwards and joined to the plate of the mesosternum. In the male a ridge extends from each side of the plate as far as the fourth segment."

The following is their detailed description:

ACANTHIA LECTULARIA.

"Red-brown, clothed with fine short yellowish hairs, and generally covered with white dust.

Head and *pronotum* finely punctured. *Elytra* with large deep distinct punctures.

Abdomen mottled with black; above perceptibly crenate-punctate, beneath shagreened."

The black mottling of the abdomen is, in the writer's experience, due to faecal specks, and is not inherent in the chitin as no trace of it can be seen in cleared specimens.

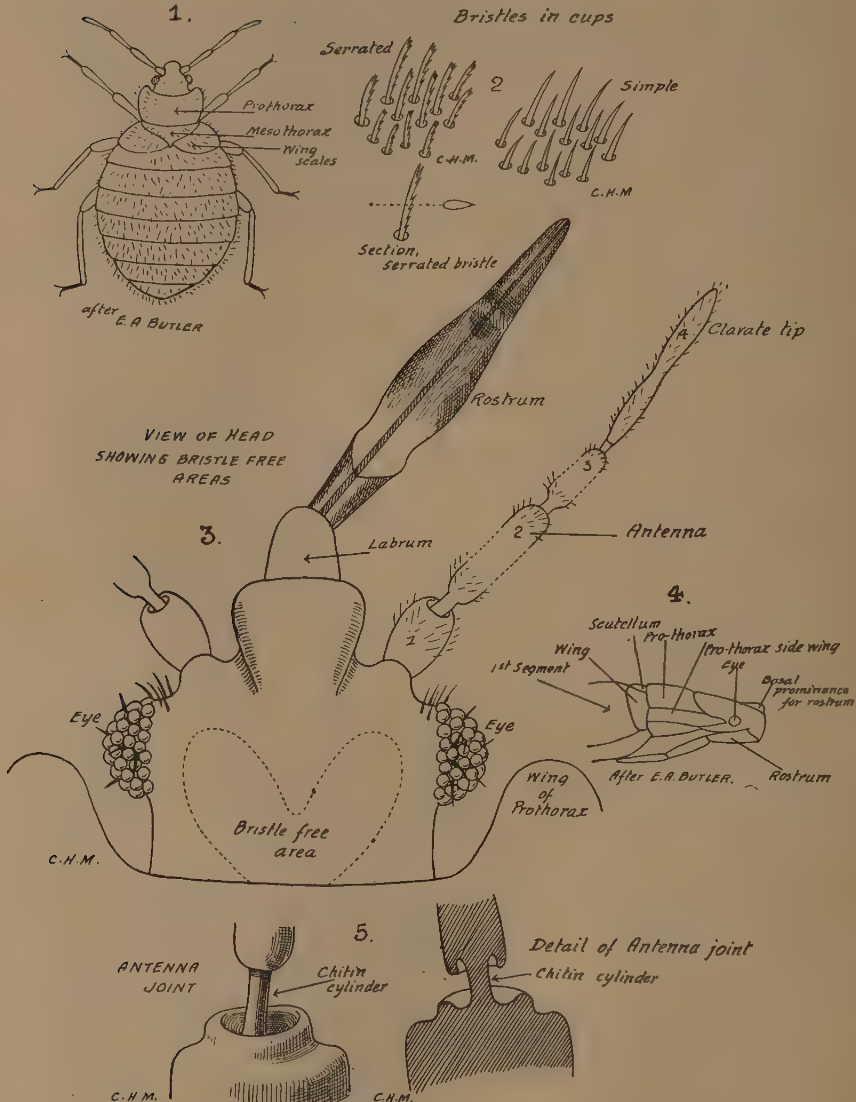
External Anatomy.

In general appearance the bed bug is small, flattened and bristly, about 5 mm. long and 3 mm. broad (Fig. 1). Both shape and appearance, however, vary according to the period which has elapsed since the last meal. The insect when famished being more or less circular and flat, while when fully fed it both lengthens and thickens. A fasting adult is of a dull amber to chestnut brown, but when fed it shows glistening bands of a highly polished brown, modified of course by the colour of the blood ingested. These bands are due to the stretching of the bug, which exposes the underlap of the segments, this being smooth and not rugose like the portions which are ordinarily exposed. In colour the creature varies from light straw when immature, to dark chestnut brown when adult; and as commonly met with, it is often thickly incrusted with dirt which has caked on its surface, the dirt being faecal matter evidently deposited on it by others, owing to their habit of huddling together in the darkest corners. They are often found

looking almost black with a chestnut coloured rim round them, this being due to their digestive juices turning the blood which they have absorbed into a very dense material almost like lamp-black. When the bug is found looking clear, and with little black in the alimentary canal, it can at once be put down as not having had a meal for a long time. It seems to have a marvellous power of doing without food, having been kept by Westwood for twelve months sealed up in a bottle, and the writer has kept one, after a full meal, for over six months in a pill-box. It, however, makes full use of its opportunities when they offer, should the previous meal be digested.

Messrs Howard and Clark of America, in their experiments on insect transmission of the virus of Poliomyelitis (*Journ. Exper. Med.* xvi. No. 6), kept the bed bug in captivity, and found that one meal must be digested ere the bug could be induced to take another, and "hence four or five days to a week passed between successive feedings." The "feedings" spoken of must have been meagre, as the writer's own experience is that, when the insect is allowed to reach repletion, the fasting intervals are lengthened to a great extent. In unoccupied houses there must be very long periods without food, and as the creature is usually found hiding in cracks and crevices to escape the light, this may have started the not uncommon popular belief that it actually feeds on wood. At present we do not know what, if anything, it feeds on when blood is not available.

Macroscopically the bug is seen to be divided into two distinct parts, one, the smaller, comprising head and pro-thorax, while the other and larger part, includes meso- and meta-thorax and abdomen. The meta-thorax, however, is not visible from above, being covered by the rudiments of the front pair of wings which persist as two small scales at the base of the abdomen. The head is closely surrounded by the pro-thorax, whose anterior lateral borders project almost as far forwards as the line of the eyes, so that the head appears as if sunk in the thorax. The meso- and meta-thorax and the abdomen taken together form almost a circle, which in the male is distinctly pointed at the anal end, while in the female, the circumference is hardly broken at all. The general surface of the whole creature is covered with bristles which rise from cup-shaped depressions with perforated bases (Plate XXIII and Fig. 2). The bristle fits into the perforation, and muscles are attached to its base so that the bristles can be erected when occasion demands, as was once observed by the writer when one bug met another. Ordinarily, however, they lie pointing backward.



Figs. 1—5 Details of the external anatomy of *A. lectularia*.

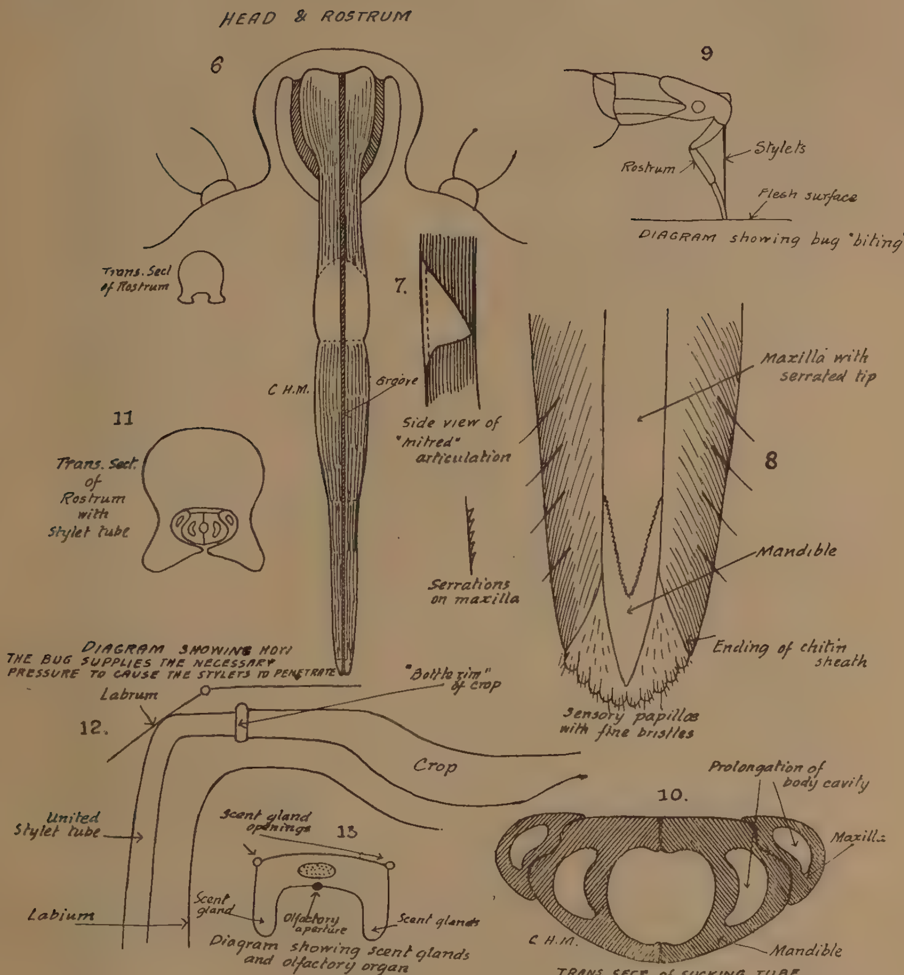
On the ventral surface the cup depressions are very much smaller than on the dorsal, giving, in fact, little more than room for the base of the bristles which are themselves rather finer. Those on the dorsal surface are very conspicuous indeed as may be seen from the accompanying photograph (Plate XXIII, Fig. 1) taken of the exoskeleton after boiling in caustic potash. The white spots, seen mostly on the second segment, are holes left by bristles which have fallen out. The bristles seem to be very fragile, as in almost every bug which was examined many were found snapped straight across, this being possibly a sign of age, like the tattered and worn condition of a bee's wing. Among the bristles there are two varieties, the one simple, like hedgehog spines, while the other is stouter, serrated up one edge, and ending at the apex in one or more spikes, usually three. These serrated bristles, which are slightly curved, have a cross section similar to that of a razor blade, the thin edge being serrated (Fig. 2). The simple spines are greatly outnumbered by the serrated, and are always arranged more or less regularly, while the serrated do not seem to follow any particular plan. On the antennae both kinds appear, the two apical joints having long simple pointed ones arranged in straight lines, and usually in pairs, while the two basal joints bear the serrated variety with spiked tops (Fig. 3). The basal joint is thickly supplied with bristles at its apex, and to about half way down, but is practically destitute of any on the proximal half. On the labium, but not on its first joint, the bristles are simple and arranged in pairs all pointing to the tip of the organ, while elsewhere on the head are found the spiked serrated kind. Round the profile of the head viewed from the dorsal surface, the bristles are rather stouter and more serrated than elsewhere. Above the eye there is a collection of a few longer serrated bristles, and one or two on the eye itself. On the dorsal surface of the head, as shown in the sketch, is a bristle-free area (Fig. 3). This marks the locations of the attachments of the muscles which work the pharyngeal pump.

The under surface of the head is clothed rather more sparsely with bristles of the serrated type alone. These are arranged mostly at the edges, leaving the middle line where the proboscis lies when at rest, bristle free, and this clear space is continued along the ventral surface of the thorax which is grooved for the reception of the proboscis. The thorax otherwise is thickly covered with bristles.

The legs, Figs. 19 and 20 (which will be treated in detail later on), bear both kinds of bristle, the simple being on the inner surface; there are also at the apex of each tibia several very stout short spines, as

well as a "brush" formed of many fine bristles. These "brushes," which are never seen on an immature specimen, may be cleaning organs of some sort, but it seems quite as likely that they help the bug to attach itself to rough surfaces. (It is very difficult to shake a bug out of a paper pill-box, an effect possibly due to the presence of these "brushes.") The abdomen is supplied with both kinds of bristle, the simple being on the under surface and in the middle line. In the female the anal segments have a preponderance of the simple with very few serrated, while in the male the numbers of the two kinds are about equal.

The shape of the head is best seen in the accompanying sketches (Figs. 3 and 4) taken dorsally and also laterally. On either side of the median prominence, from which the rostrum rises, are the antennae, composed of four joints. The first is short and stumpy, rising from a slight knob, called by Douglas and Scott the antenniferous process, situated midway between the median prominence and the eye. The second is long and rather thicker at the apex than at the base. In length the third joint equals the second, but is rather more slender, and the chitin is much thinner, while the fourth joint, about two-thirds the length of the second, is slightly clavate at the apex. A very noticeable point about the antenna is that, although composed of only four joints, it has a great range of movement, due to the nature of the articulations which give the effect of a double ball and socket joint (Fig. 5). The end of each joint taking part in any articulation, is slightly narrowed and hollowed out. Connecting these two sockets is a short chitinous cylinder which has very free movement in each, and thus the antenna having three such articulations can be moved in any direction. As already mentioned, they are well supplied with bristles of the two kinds. The presence of only simple bristles on the two slender apical joints, would suggest that their function is sensory, although the presence of similar ones on the ventral surface of the legs can hardly be explained on this ground. The rostrum of the bed bug (Figs. 3 and 6) is a three-jointed grooved organ which, in life, is usually pressed up against the ventral surfaces of the head and thorax, these being grooved to receive it. Morphologically it is the labium elongated to form a projecting sheath for the mandibles and maxillae found in the groove running down the length of the rostrum. The position of this groove is such that were the proboscis to be projected horizontally forward, it would lie on the dorsal surface of the organ. As with the rest of the body surface, the rostrum is plentifully



Figs. 6—13. The mouth-parts of *A. lectularia*.

supplied with bristles which here are simple, and arranged more or less in pairs as regards their position on the organ, and all pointing to the tip. Since it acts as a sheath, it is formed of fairly stout chitin, with the exception of the extreme tip, which is covered with sensory papillae and has on its surface a few very fine pointed bristles (Fig. 8). With this delicate tip the bug locates the spot where it is to feed, and a full-fed bug, when meeting a famished one, has been seen by the writer to protrude its proboscis horizontally towards its approaching relative, though why this was done remains a mystery. Famished bugs when imprisoned under a very shallow watch glass, tested the nature of the glass with the rostrum. The tip of the proboscis encircles the stylets, and, no matter how deeply they be inserted, it always surrounds them as in the case of the mosquito.

When describing the rostrum, Landois says that its apex has "slightly-outward-turned corners." The writer believes this to be wrong, and refers to Fig. 8.

To enable the lancets to penetrate deeply, since the proboscis sheath itself does not enter the wound, the latter bends towards the bug at the articulations, and especially at the articulation between the first and second joints (Figs. 6 and 7). Here the chitin is, so to speak, "mitred" away so as to allow of the great bending made necessary by very deep penetration (Fig. 9).

Diagrammatically the relative positions of the rostrum and stylets, in relation to the head when sucking, is shown in Fig. 9.

The protecting groove is open all its length, and only where it joins the head is it covered, and here the labrum or upper lip, which is a small triangular piece articulated to the anterior dorsal surface of the median prominence of the head, covers the groove (Fig. 3).

When discussing the labium and labrum, Landois gives each an extra joint, and thus makes the former have four joints, and the latter two. Probably this had been due to an idiosyncrasy of his "School" of Zoology, as the joints can be seen very clearly.

Inside the groove of the rostrum, as has already been said, are to be found the lancets (Fig. 11). These are composed of the mandibles and maxillae which have been transformed into long needle-shaped organs beautifully adapted for piercing, and united together to form a tube for the conveyance of the blood on which the bug is to feed (Fig. 10). Of the four component parts of this tube the maxillae are the most delicate. They alone are serrated, and for only a short distance at their tips, the serrations being so placed that they act as barbs when

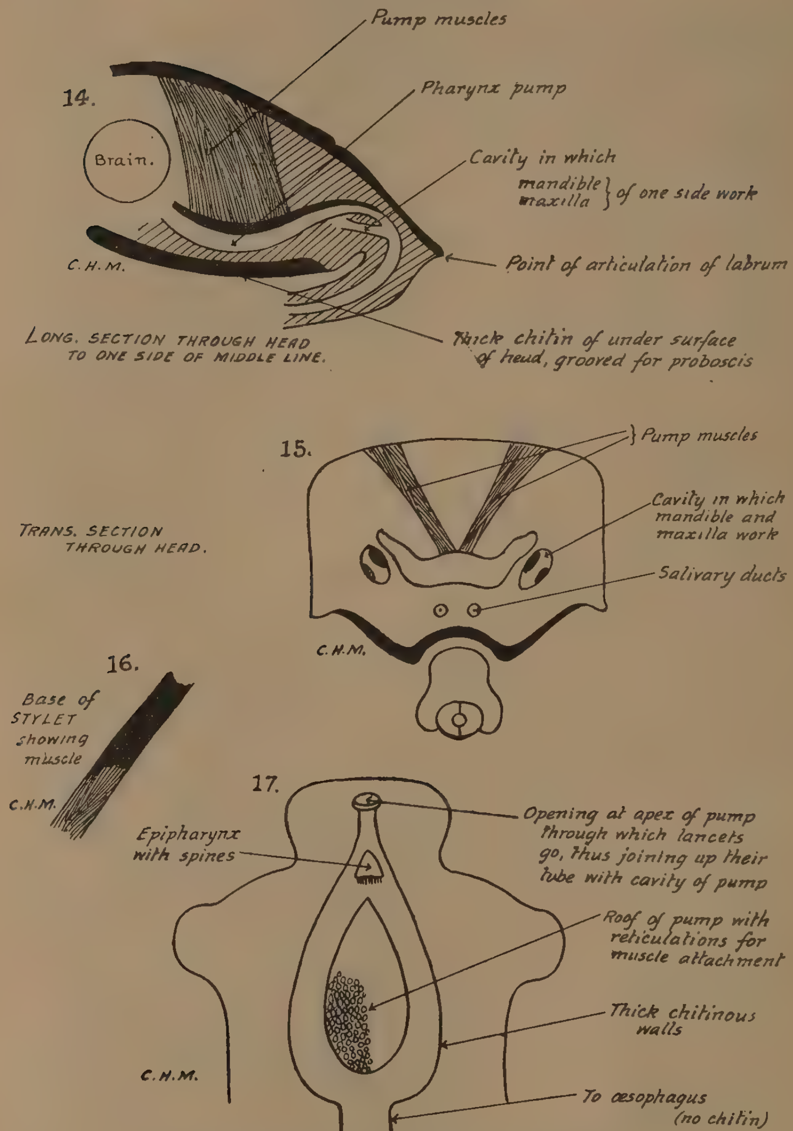
the lancets are withdrawn. There is no trace whatever of palps belonging to the maxillae. The two mandibles unite to form an organ with a sharp piercing point, and a hollow interior which serves as a sucking tube. When at rest in the proboscis groove, the mandibles project rather further than the maxillae as shown in the sketch (Fig. 8) of the enlarged tip of the rostrum.

The mandibles are not toothed, and evidently in the process of feeding *they* first pierce the skin, and afterwards the maxillae, with their barbs, further lacerate the tissues and start the flow of blood which is sucked up by the mandibular tube. A section across the stylets and rostrum shows the two mandibles united together with a maxilla on either side (Fig. 10). The same arrangement continues to the tip, except that the maxillae become relatively smaller as they recede from the base. All four stylets have cavities in their substance quite apart from that which they unitedly form, these being prolongations of the body cavity.

Landois' statement that each of the components of the stylet tube forms a quarter circle, must be put down as an error, due to his lack of suitable appliances for section-making.

In a mandibulate insect, such as a beetle, the mouth parts (the mandibles and maxillae) are outside the mouth proper, so that mastication takes place before the food enters the mouth. In the bed bug it would at first appear as if these parts were inside the mouth and not in their normal position, but sections of the head show that they work in cavities which communicate directly with the outer air. These cavities open to the exterior at the base of the rostrum, and are continued until they join up with the hollow which becomes the groove in the rostrum. The sketches show what is meant (Figs. 14 and 15), the longitudinal one being taken a little to the side of the middle line, while the transverse one is taken almost where the lancets have their muscle attachments. This point is just beyond where the pump muscles end, and is therefore about the level of the brain.

The sketch (Fig. 17) shows a dissection of the head and the relative position of the pharyngeal pump (of which more hereafter) and the mouth parts. The lancets unite, and the tube thus formed from the maxillae and mandibles passes through a chitin-surrounded orifice, in the front of the crop, likened very appropriately by Landois to the thickened edge round the neck of a bottle (Figs. 12 and 17). This orifice is under the prominence from whose distal end the labrum arises, and is fastened to the under side of the prominence at that point. Thus the stylet



Figs. 14—17. Pharyngeal pump of *A. lectularia*.

tube connects directly with the pharynx. The mandibles and maxillae at their proximal ends are swollen and hollowed out for the insertion of their muscles (Fig. 16), called by Landois "Musculi erectoris et retractoris rostri." The chitin of the under surface of the head is very thick, especially in the middle line, forming, in fact, quite a ridge, grooved externally to hold the proboscis when not in use (Figs. 14 and 15). The upper surface is also thick, thus giving the strength required for the attachment of the muscles which work the pharynx pump, but of the two surfaces the ventral is the thicker.

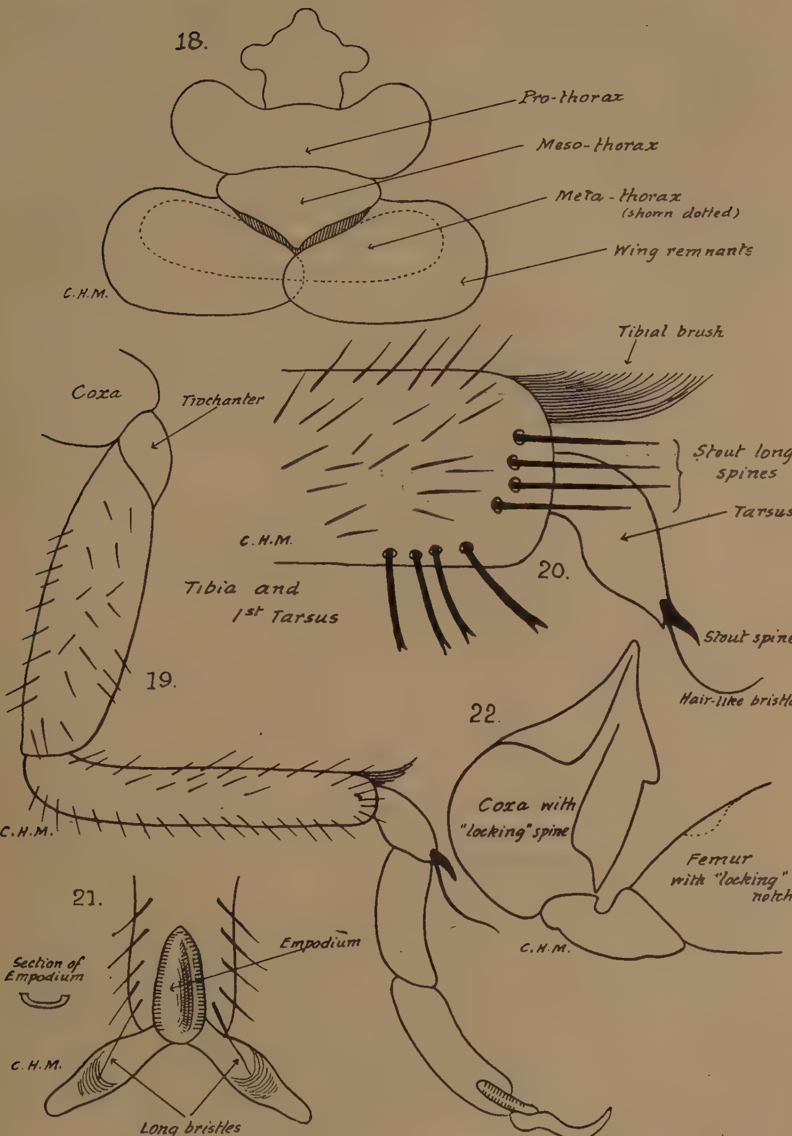
In the thorax of the bed bug (Fig. 18) the three main divisions of pro-, meso-, and meta-thorax are found, each being well developed and articulated to the others. Of the three regions, the pro-thorax is the least complex, since it consists of only the tergum and sternum. Its breadth is much greater than its length, and has broad leaf-like expansions of its chitinous covering at the sides. These run forward by the side of the head almost as far as the eyes. At the posterior border the sides curve in rather more sharply to the base line. The broadest diameter of the pro-thorax is at about one-third of its depth measured from the tips of the anterior lobes. Its surface is covered, dorsally, with bristles in their bowl-like sockets, while the chitin between the sockets is slightly rugose. The under surface is like the upper in contour and bears the coxal cavities, here very close together, leaving, in fact, just room for the tip of the proboscis. In texture the surface is similar to that of the dorsal with the exception of the lateral area at the base of the coxal cavities. Here there is a large area peppered all over with small pits. These special locations are for the attachment of the muscles of the front legs. In the middle line on the ventral surface the chitin of the pro-thorax is very much thickened. In fact it forms a strong ridge internally, whose external surface is grooved for the reception of the proboscis when at rest, and thus continues the line of the similar groove on the under surface of the head. This segment bears a pair of spiracles which are situated external to the coxal cavity and in the angle formed by it and the base line of the segment.

Viewed from above, the meso-thorax or scutellum has the appearance of a straight line from whose extremities two ogee curves arise and meet posteriorly at a point. Having a double set of articulations, namely the second pair of legs and the wings (although these last are non-functional), this section is much more strongly built, and to give the necessary room for the muscles it has well-developed pleura. These are much indented and strengthened for the muscle attachments,

being very rugose with parallel furrows whose length is almost at right angles, to the length of the bug. On its anterior lateral angles the mesosternum bears two spiracles. Their position is such that they are invisible from either the dorsal or ventral surface. The spiracles on the pro- and meso-thorax do not possess the " wax " bladders found on all the other respiratory openings.

From above, the meta-thorax is inconspicuous and its dorsal portion, being covered by the wing cases, is puncture free, and carries no bristles. The pleura, in their sculpture, strongly suggest the Astrea type of coral, in that there are central larger depressions from which others, smaller ones, radiate and anastomose with those radiating from other central depressions. Besides this, it is greatly strengthened by a broad rib which divides the episternum of the meta-thorax from the epimeron of that segment. This rib is sculptured in a manner found nowhere else on the bed bug. The part of it near the metasternum is reticulated with a band of almond-shaped papillae whose length is along the band, while, towards the meta-notum, the reticulations take on a more or less polygonal form. Besides this the chitin of the edges here is greatly thickened. Ventrally the metasternum is conspicuous and has a considerable bulk, and thus it projects somewhat over the first abdominal segment (Fig. 29). This increased bulk has the effect of separating the coxal cavities which here, as on the segment immediately preceding, are well apart. In this space between the coxal cavities of the mesosternum is to be found an organ which, from a structural point of view, would appear to possess olfactory properties. It will be treated in detail later on, but here it may be said that it lies in a cavity which opens to the exterior by a pore in the middle line of the metasternum where it overhangs the first abdominal segment. This pore cannot be seen from the ventral surface.

The legs of the bug are of the usual insect type and consist of coxa, trochanter, femur, tibia, and tarsus (Fig. 19). The latter is divided into three joints of which the first is the shortest and the third the longest. As previously mentioned, the legs are plentifully supplied with bristles of both kinds. The simple are found on the ventral surface of the tibiae, on their ends as well as on the tarsi, while the serrated occur on the outer edge and sides of the tibiae, on the femur, and on the trochanter. Besides these two kinds, the ends of the tibiae have, on their ventral surfaces, a dense brush formed of bristles of different lengths, so arranged that their tips continue the line of the ventral surface of the tibia, which is brought about by the longer ones having their tips greatly



Figs. 18—22. Structural details of *A. lectularia.*

curved (Fig. 20). At the base of this brush, and on the anterior side of the tibia, is a group of four long stout simple spines, and at the opposite side of the joint are three very strong spines blunted at the point. On the first tarsus at its apex is one stout spine from whose base springs one *very long* pointed hair or bristle, the purpose of which the writer has failed to determine (Figs. 19 and 20). The third tarsus bears the two claws and the empodium (Fig. 21), on whose surface are lateral ridges which evidently serve to increase the bug's foothold. In the bee or the house fly the empodium enables it to walk suspended from glass. This the bug cannot do, in fact it has difficulty in walking at all on glass. The terminal claws are stout, strong, and well adapted for keeping a tight hold. To further enable the insect to obtain a good grip, the coxae have on each outer surface a stout prominence which fits into a corresponding depression on the femur, so that the creature when it holds fast to anything can, so to speak, "lock" its legs and thus render itself rigid (Fig. 22).

According to Douglas and Scott, "The abdomen of the RHYNCHOTA in its greatest development consists of nine segments...of which only the first six are proper abdominal segments, the last three...differ in form more or less from the others, and subserve the functions of the sexual organs. (In some families) we find apparently seven abdominal segments, as the first genital segment retains entirely the form of an abdominal segment."

The writer has done no comparative anatomy of the RHYNCHOTA, and hence is not in a position to give the segmental homology of the various parts seen in the two sexes of bed bug, so he contents himself with merely describing them.

The only peculiarity in the abdomen of the bug common to both sexes, is that the first four segments are different from the other three, as they bear in the middle line on the ventral surface a broad shallow groove formed by a slight invagination of the chitin, and well supplied with bristles. This portion, when the bug begins to feed, is the first to respond to the internal pressure and evaginate. Continued feeding and consequent lengthening brings the underlap of the segments into view, as already mentioned. This groove seems to be what Messrs Douglas and Scott refer to as the ridge which "extends from each side...as far as the fourth segment." The writer finds the groove to be present in both sexes, and not only in the male as held by them.

Each of the abdominal segments bears a couple of spiracles on the

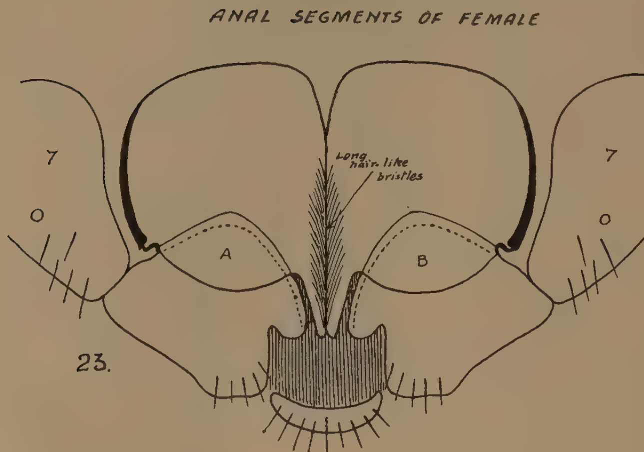
under surface near to its outer edge and on the dividing line between the bristly rugose part and the shining smooth underlap.

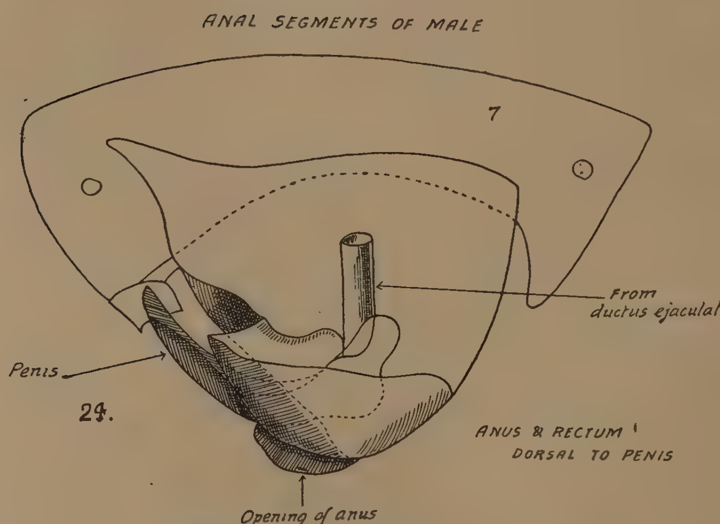
The first seven abdominal segments of the male bug are quite normal, but, in the female, on the ventral surface of segment four, and to the right of the median line, at the posterior edge of the segment, is found the opening to what is called the "Organ of Berlese." This is a round mass of a dull white colour lying in the body cavity at the right side. Under it is found a layer of fine chitinous rods, but what are the functions of the organ the writer hesitates to say, the authorities differing greatly on the subject. Berlese, its discoverer, says that its use is to enable the female to derive nutriment from excess of spermatozoa received in copulation, while Ribaga infers from the presence of the chitinous rods, that it is a stridulating organ, although he comments on the peculiarity that it is found only in the female.

The genital segments of the female bug are composed of pairs of chitinous plates which are well supplied with simple bristles. Following the sixth segment there are four plates, two of which, meeting in the middle line, form the vagina, which is a groove practically thatched with long whip-like simple bristles which interlace (Fig. 23). Outside these are a pair of plates one on either side, each bearing a spiracle, and being in all probability part at least of the seventh abdominal segment.

The two central plates have their lateral edges strongly reflexed and thickened, evidently to secure strength. In shape they are approximately quadrilateral except in the middle line, and at the posterior end, where they are prolonged into blunt points and thus separate the remaining pair of plates. These last are overlapped in part (portions *A* and *B* in Fig. 23) by the central plates, and at their anterior lateral edges meet the spiracle-bearing portions of the seventh segment. These overlapped plates are more or less quadrilateral, but in the middle line and posteriorly there is a large notch which leaves a space lined with delicate chitin and closed by an unpaired chitinous cap, often seen reflexed in specimens which have been mounted in balsam. This is where the anus opens, and it would appear to be covered by this lid. Around the anus, as previously mentioned, the bristles are very long and all simple with the exception of those on the anal cap, and here they are all serrated. Segments six and seven have also a preponderance of the simple with the serrated at the edge.

In the male there is a great asymmetry in the genital segments. The seventh is quite normal, but the following one, as shown in the





Figs. 23, 24. Anal segments of *A lectularia*.

sketch, is much less in breadth, and instead of being flattened is rather conical (Fig. 24). This segment has, at the left, a very deep lateral notch in which the major part of the penis lies concealed. Since this organ projects forwards so as to overlap segment seven, the posterior left lateral corner of that segment has been recessed to receive its tip. The structure of the penis will be dealt with when describing the male reproductive organs.

The anus of the male is surrounded by a chitinous ring and is slightly to the left in position, and not in the middle line.

The male bug has its anal bristles made up of the two types, the simple being outnumbered by the serrated. Here, as in the female, the bristles are much longer than elsewhere, the serrated being specially robust.

Alimentary System.

When the bug is about to feed it walks in a very peculiar jerky manner, quite different from its usual method of progression. and carries the proboscis at right angles to the body, evidently using the sensory tip to locate a suitable spot, on finding which, it begins to drive the stylets into the flesh. Reasoning from a structural point of view the writer believes that the following is possibly the method by which the stylets are made to penetrate. The head is alternately moved close to, and further away from the skin, and the insect is seen to tremble all over until evidently the suctorial tube has reached the required depth. As the united mandibles project at the tip a little beyond the maxillae (Fig. 8), *they* would first pierce the flesh. During the downward pressure which, from the trembling referred to, would seem to be relatively great, the stylet tube will be hard up against the " bottle-rim " chitinous ring at the apex of the crop, while the labrum, being pressed downwards, will prevent the stylets from springing at the bend (Fig. 12). Thus the power is applied vertically, and so the mandibles are forced through the skin. Possibly they may be helped by a see-saw movement similar to that of the maxillae, which are toothed in such a way as to keep the tip from being easily withdrawn (Fig. 8), although there is not much resistance to their insertion. Assuming that the mandibles work alternately, and a little ahead of the maxillae, the wound would thus be deepened for further penetration by the maxillae. There would seem to be some evidence that the sucking action of the crop commences early in the operation, as air can be seen *through the chitin of the body wall* to collect in the stomach, and this points to suction

having arisen before there was fluid to imbibe. It would also appear that the suction is spasmodic, as the air can be *seen* collecting as separate bubbles.

Landois states that the maxillae are unequal in length. The writer finds that they are equal, and suggests that the error may have been due to the relative positions of the maxillae, after the alternate movement, on withdrawal.

In describing the method of inserting the stylet tube Landois says:

"Die Bewegung des Stechrohres ist zunächst eine mittelbare, indem bei Hebung und Senkung der Unterlippe, in deren Rinne es belegen ist eine gleiche Bewegung der ersteren erfolgt" (Part 1, page 209, lines 4, 3, 2 from foot). On the next page, however, he says:

"Während des Stechens bleibt die Unterlippe unbeweglich liegen" (Part 1, page 210, lines 22–23).

The writer is at a loss to say what Landois really meant.

Even under ideal conditions for feeding, the bug does not always settle down at once to enjoy its meal. On one particular occasion observed by the writer, the lancets were inserted nine times in different places before the insect was satisfied with the spot. When finally suited and at work, the feeding process took about eight minutes, the glistening bands beginning to appear at the end of four. The first sign of satiety is a curious jerking of the antennae, then, the sucking tube being smartly withdrawn, the bug makes off with considerable speed, the rate being much the same whether full or famished.

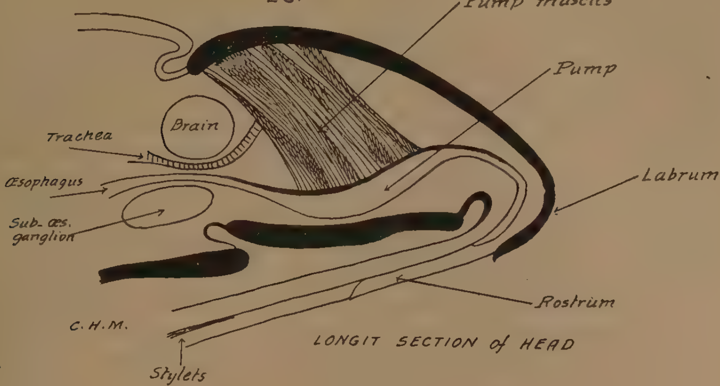
Measurements taken before and after feeding, show that the greatest increase in bulk is in the dorsal to ventral direction.

Before	After
Length 5 mm.	6 mm.
Breadth 3 mm.	3 mm.
Thickness 0·5 mm.	2 mm.

In the process of feeding there is no external sign of pumping, the head chitin being so thick that it conceals all movement, although the striated pump muscles are very strong and in bulk fill up the major part of the head. As previously mentioned, they are fastened to the bristle-free area of the upper surface of the head (Fig. 3). Their ventral ends are fastened to the dorsal surface of the "pump," *i.e.* crop (Figs. 14 and 15). This organ is a pear-shaped chitinous swelling of the pharynx, which at the anterior end communicates directly with the tube formed by the lancets, while posteriorly it leads into the oesophagus.

From front to back the pump is curved after the ogee pattern (Fig. 26), the anterior end being the higher, nearly reaching the dorsal surface of the head, while, at the hinder end, it rests directly on the thickened

26.



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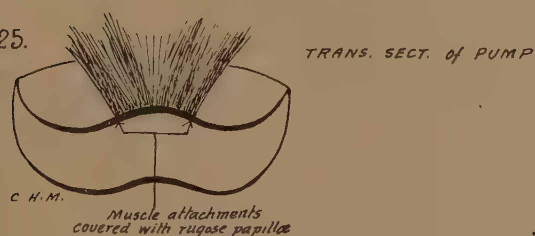

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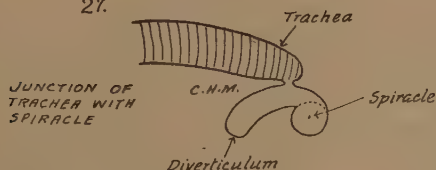


Figs. 25—27. Pharyngeal pump of *A. lectularia*.

ridge of chitin on the under surface of the head. From side to side the pump in section is more or less like the letter U, the sides being very short, while the base is very much greater in proportion (Fig. 25).

Landois acknowledges that he cannot fix any distinct function for the crop, and he definitely says that it is "not suited for creating suction on account of its strong sides." Microtome sections would have showed him his error. He suggests that peristalsis of the gut causes the suction, but the great tenuity of its wall rules this out of court.

The part of the crop to which are attached the muscles which operate the pump is reticulated very irregularly with lozenge-shaped papillae, the surface of each papilla being very coarsely rugose (Fig. 17). On the dorsal surface, and indeed helping to form part of its roof, and immediately at the base of the labium just where the "pump" broadens out, is the epipharynx. This is a triangular piece of chitin, the apex pointing to the anterior end. On its posterior border there is a row of very short delicate spines, about ten in number, which hang down into the cavity. When the dorsal surface of the crop is dissected off (after having been boiled in caustic potash) and mounted for microscopic examination, the spines of the epipharynx are seen quite clearly on a horizontal plane (Fig. 17). Since in a microtomed bug they are seen hanging down into the pharyngeal cavity, it would certainly suggest that they are very loosely articulated to the chitin plate. The writer can offer no suggestion as to the function of these spines.

Landois says that besides the epipharynx there are four other smaller chitin plates on the dorsal surface.

The writer, after many dissections, would suggest that these "chitinous plates" are merely an optical effect caused by the remains of the attachments of the muscles which move the crop-roof.

The pump needs no muscular effort to bring it back to its at rest position on account of its shape and the elasticity of the walls which are formed of stout chitin (Fig. 25).

The function of the crop in the bug being solely that of providing the suction necessary for the imbibing of blood, it cannot therefore be used, as in other insects, for purposes of storage. Thus ingested blood goes directly into the stomach, and after that is filled, the first portion of the intestine is utilised. The stomach and intestine have irregularly disposed muscle bands in their walls (Fig. 28). When a bug has been allowed to have only a small meal and is then dissected, the stomach usually appears to be symmetrical and laterally lobed, or occasionally of an asymmetrical shape depending on the volume of blood which has been imbibed. When the stomach is quite full there is no sign of lobes at all, but (if used for storing) the first portion of the intestine then shows the unilateral pocketing (Fig. 28). The writer has

noticed that frequently, but not always, the bug stores blood in the anterior end of the intestine long before the stomach is full. In nearly every case when a partially fed bug was dissected, it was found that, although some of the blood imbibed had been stored in the first portion of the intestine, there was a clear empty space immediately after the stomach separating the blood in the stomach from that in the intestine. This is due to a sphincter which, one would be tempted to think, must be overstrained when the bug feeds to repletion, as the junction between stomach and intestine is then not clearly defined. It is quite possible that the effect is the result of the peristaltic action of the alimentary tract forcing the blood onward in portions, while the sphincter may not begin to act until there is some slight strain on it. Considering the suction that must be created when the pump pulls, it is rather curious that there is no trace of a mechanical valve between the pump and the stomach to prevent regurgitation of what has already been imbibed, there being no sphincter muscle between pump and oesophagus. The function of a valve may, however, be undertaken by the very thin-walled oesophagus being occluded by the pressure of the surrounding tissues when the pump sucks, or it may be that the muscles work peristaltically from anterior to posterior, and the see-saw movement of the roof of the pump may thus act as a valve which is alternately closed and opened. In favour of the first suggestion is the fact that the stomach always contains some air, which expanding under the reduced pressure would tend to occlude the thin-walled oesophagus. On dissecting a bug under fluid, the stomach always becomes very prominent when the dorsal body wall is opened, because of the air contained in it. Among the Diptera large volumes of air are also found in the alimentary tract, though the purpose is not apparent. In the bug, the presence of this air would seem to be due to the creature commencing to work the pump ere the lancets were imbedded, or else continuing to work it after they were withdrawn. As the bug imbibes blood it is mixed with saliva from the salivary receptacles. These are two in number and lie on the dorsal surface of the thorax on either side of the oesophagus just before it branches out into the crop. When freshly dissected out, the colour is found to vary in different specimens. Landois, in speaking of these receptacles, calls them the "large spherical salivary glands," and says they are of an exquisite "yellow green" colour. In the writer's experience the colour varies very much, but never has he seen them "yellow green." Whatever be the nature of the colour it is soluble

in water, and is brightest in a famished specimen. On dissecting a bug "dry" the receptacles were seen without microscopic aid as two blood-red or crimson specks, while dissecting under water they are found to vary in colour from terra-cotta to pearly white, depending on the time which has elapsed before they are laid bare. Should they be found coloured terra-cotta, they soon lose the tint and become pearly white.

Besides varying in colour, the receptacles vary in form, being when full shaped like an egg and, when partially emptied, more like a pear (when pear-shaped the duct leads out from the broad end), but in no specimens of the scores examined has the writer found them spherical. Landois calls them "kugelförmigen" in the text, yet he figures them as more or less egg-shaped. (For his figure refer to the plates illustrating his article, Plate XXII, fig. 1.)

Leading from the receptacle is a thick-walled duct (·02 mm.) with a very narrow lumen (·002 mm.). This duct after leaving the receptacle divides almost at once into two, each rather more slender than the parent duct. The lumen in these is similarly rather finer than in the short parent length. This latter slightly tapers from its opening into the reservoir down to where it bifurcates, and at its inner opening slightly bells out, forming a funnel. Considering their size the ducts are very strong, and persist through the precarious operation of separating them out. Possibly this strength is due to the lining membrane of the duct being strongly chitinised. When the duct happens to be broken, this lining often protrudes from the end. Landois says that the inner coating of the duct is transversely marked and recalls a trachea thread. This is true to a certain extent, but only under oil immersion magnification is the effect seen, and then but feebly.

The two daughter ducts have different destinations. One goes forward to the head, and meeting the companion one from the other side runs parallel with it and under the pump (Fig. 15) till they end at the base of the labium where they discharge. The other goes forward a little way then bends round, and running back, leads into the salivary gland of its side (Fig. 28). Landois calls these the "small spherical salivary glands," and the name is very appropriate. They are situated, one on either side at the anterior lateral region of the stomach, and are fastened to it by a group of delicate muscle bundles. The ducts leading from them enlarge slightly and gradually, between the gland and the receptacle. Landois appears to have confused between these, as he was evidently not able to trace the connection between the two,

and thus they had appeared to him as two separate pairs of glands, although Dufour suggested that one pair perhaps acted as a reservoir.

(The saliva of the bug is said to be the cause of the swelling which occurs when a victim has been " bitten." The writer's own experience is that there was no discomfort whatever when the lancets were being inserted (except in one case which may have been caused by the stylets

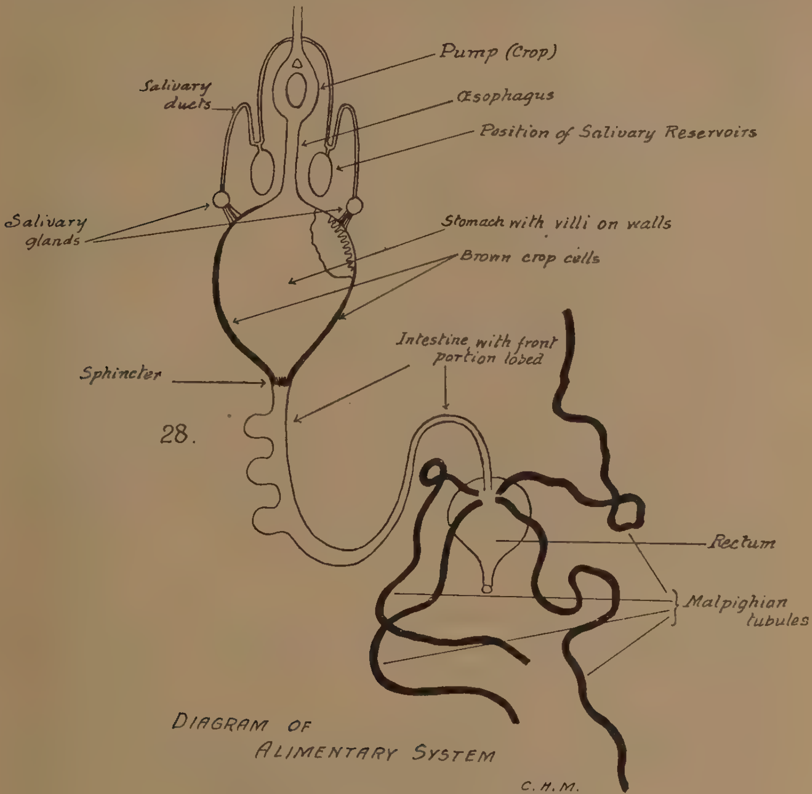


Fig. 28. Alimentary canal of *A. lectularia.*

touching a small surface nerve) and there was no after discomfort even with a " bite " of five minutes' duration unless the place was rubbed. A single rubbing suffices to start the irritation, and the marks remain visible for about a fortnight; the last sign to disappear being a red mark showing the exact spot where the lancets had penetrated.)

After leaving the pharyngeal pump, the oesophagus becomes very narrow and accompanied by a trachea trunk on either side passes through the circum-oesophageal commissure (Fig. 26).

Between the end of the crop and the beginning of the stomach, the oesophagus gradually widens until, as the stomach, it broadens out considerably. The tissues forming the stomach walls are very elastic and have bands of muscle fibres in their substance. One effect of these muscles is to alter its shape, as has already been mentioned. When full to repletion, the stomach stretches from side to side of the abdomen, while under starvation conditions it is more or less confined to the middle of the space. Repletion makes the stomach pear-shaped, the pointed end being at the junction between stomach and intestine.

Repletion always carries with it the presence of much air in the stomach, while even a hungry bug has some evidently left over from last meal. Once the blood is in the stomach, digestion begins; the first visible result being that the colour changes from red to sepia. This is brought about by the secretion of the local glands. The position of these is best described by saying that they are arranged on the walls of the pouches of the stomach, the pouches being on a horizontal plane. When fully fed the position alters because of the stretching of the walls. These glands are of a very dark brown colour. At the apex of the stomach they are sparsely placed and light in colour, while at the base, where is the sphincter between stomach and intestine, they are much more numerous and darker (Fig. 28); in fact the stomach would here seem to have a dark brown continuous band on it. These glands evidently secrete a sepia-coloured fluid, but its nature is at present unknown.

The effect on the blood is exactly as Landois describes, *viz.* the absorbed blood is changed to a black or brownish-black mass in which are many small dark brown granules. What these granules are the writer cannot say.

A great deal of the digestion must be done in the stomach, for its walls and sides, besides being themselves very glandular, are covered with villi, so that there is available a large area of glandular tissue. At the posterior end there is the sphincter muscle which closes the passage to the intestine. This latter lies curved to the left in a plane at right angles to the plane of the stomach and surrounding its base. Occasionally the intestine is pouched asymmetrically but all on one edge, and not irregularly over the whole surface. One curious thing about the intestine is that, when utilised for storage, as happens when

the bug has been allowed to feed without hindrance, the blood is occasionally seen to be filling the pouches above mentioned, while between each two " pouchfuls " there is a short space of intestine with no blood or other contents. In one case there were seen three such collections between which there were no contents. the walls of the intestine being quite occluded. This may be due to the bug having to make the most of its opportunities for feeding, and when the stomach is full, having to utilise the anterior portion of the intestine for storage. It is quite possible, however, that the condition may be a result of death throes, as the bugs were chloroformed soon after their meal, but the writer thought it well to put the fact on record. The posterior end has no pouches, is very much thinner than the other, and the contents, when present. are of the afore-mentioned sepia colour.

Next comes the rectum, the junction between which and the intestine bears the Malpighian tubules which are four in number. They are individually longer than the whole alimentary tract, being indeed from one and a half to twice the body length. The whole alimentary tract in a famished bug is three times the body length, while in a fed one it is more than twice. Probably the difference due to feeding accounts for Landois' observation that the alimentary tract is not twice the length of the body (Fig. 28).

The rectum is practically always full of dense dark sepia-coloured contents, of a texture strongly resembling lamp-black. Sometimes, however, the colour is of a light brownish-yellow, in which case the dark granules are not present. This difference does not seem to depend on sex, and is the exception rather than the rule, so possibly it may be a pathological condition.

The rectum is heart-shaped and opens to the exterior at the apex of the body in both sexes. In the male the anus is slightly to the left of the middle line. In the immature bug it opens on the ventral surface. If the contents of the rectum be examined microscopically, the first thing noticed is the presence of innumerable whole blood corpuscles mixed up with portions of others, the whole mass being coloured brown and containing fine brown granules. Dilution of the contents shows that the whole corpuscles still retain their normal life colour. A bug which had not been fed for at least three weeks, and whose rectum was the only opaque portion of its alimentary system, got an unrestricted meal. After feeding it ran about on a sheet of paper, and very shortly " backed " unexpectedly and passed two small drops of faecal matter. These were fixed, and on being examined under the microscope were

seen to be largely composed of whole blood corpuscles. Considering the time that must have elapsed since the previous meal, there would seem to be a power of delaying digestion as a defence against prolonged famine. A somewhat similar observed case was one of a flea which the writer experimentally fed, giving absolute freedom as regards time. After feeding for about ten minutes under a glass, it suddenly shot out a speck of black faecal matter which was soon followed by a drop of dark red fluid, and then by a drop of what looked like blood. Probably the bug in question would have more completely absorbed the contents of the rectum if food had been still longer withheld, but when a fresh supply became available it made room for it by at once clearing out the old store.

Circulatory System.

The circulatory system of the bug is of a very simple order even amongst insects, where it has been reduced almost to a minimum. There is no complex of ramifying vessels to convey the blood, no trace even of the diaphragms which in others, such as the bee, divide the system into regions and supply motive force to the fluid. There is only one vessel, the dorsal aorta, which merges so gradually into the heart that it would be difficult to indicate the point of division. On opening a bug from the dorsal surface, the almost transparent heart is seen lying in the middle line. This organ stretches from the anal end right forward to the head, and ends there just behind the brain. It is closed posteriorly, and is slightly pouched on segments five and six. These pouches are the heart chambers, and contain the ostia or valves on their sides. The organ being very muscular and contracting peristaltically from behind forwards, the blood is driven out at the anterior end, and oozing through the spaces between the various organs it completes the circuit, ultimately reaching the slight pericardial spaces where the cycle is recommenced. Since the blood percolates through the interstices between the various organs, the Malpighian tubules are thus constantly bathed in the fluid, extracting the waste matter and discharging it into the rectum. At the posterior end, and also at the level of segment three, there are a couple of thin strands of muscle fibres. These moor the heart, and would seem to be the remnants of the diaphragms found in insects. There is a slight taper from the posterior to the anterior end of the heart, and throughout its length it is closely surrounded with cells. In segment three, there is rather a

large collection of these into which goes a tracheal trunk from each of the dorsal vessels. The function of these cells surrounding the heart in insects does not appear to be known, although in the bug at least they would appear to be in the nature of fat bodies since they are very inconspicuous in a famished specimen, whereas in a well-fed one they are clearly seen.

Respiratory System.

The respiratory system of the bug is more or less of the ordinary insect type. As already mentioned, there are nine pairs of spiracles, seven on the abdomen and one each between pro- and meso-thorax, and meso- and meta-thorax.

Round each spiracle the chitin is much thicker than elsewhere and the opening is narrow in the thickness of the wall, so that the hole is of hour-glass shape.

The trachea does not rise directly from the inside of the spiracle, but from a diverticulum like a glove finger. Possibly the presence of this diverticulum, which renders difficult the passage of foreign bodies into the trachea, explains why there is no apparatus for closing the spiracle, as is so common in the insect world (Fig. 27).

The main trunk rising from this vestibule is at first slightly swollen, this being possibly a remnant of a system of air sacs, such as are found in flying insects, since the bed bug is evidently a degenerate flier.

Very near the spiracle the trachea divides, one branch going directly across to, and anastomosing with, the corresponding branch from the opposite spiracle, and on the way giving off branches on either side. The other branch, with a good deal of "slack," joins the main longitudinal trunk of its own side. These lateral trunks run from the seventh abdominal spiracle right up to the head, where, after passing through the circum-oesophageal commissure, in company with the oesophagus, they divide into capillaries in the substance of the head supplying all the organs, even the tips of the antennae and the rostrum getting their share.

Since the bug is about 0·5 mm. thick when hungry, and when full-fed increases to 3 mm., it can readily be seen that there is great necessity for the above-mentioned "slack" since the longitudinal trunks are slightly "tacked" to the dorsal surface of the body wall, and follow its movements.

In structure the tracheae are internally strengthened with the typical chitin rings, although the diverticulum connecting the trachea with the

spiracle is not so reinforced. This diverticulum is an invagination of the chitin of the ventral surface, and is so immovably fastened to the spiracle, that prolonged boiling in caustic potash has no effect on it. In other Hemiptera its function is thought to be to keep water from entering the trachea, as it is often found filled more or less with wax. In the bed bug it is empty, the creature's manner of life being such that moisture will very seldom reach it, and even should it be immersed in water the oily nature of its surface would keep it from getting wet.

For maintaining air currents through the tracheae there are several sets of muscles. Joining up each segment with the next there are longitudinal bands on the dorsal and ventral surfaces of the bug. They run from the middle of one segment to the middle of the adjoining one, and their action, on contracting, would be to reduce the length of the creature and thus drive out the air. On every segment, a little to the inside of the spiracle and arising at the division between the bristly and polished portions, are other sets. These are composed of separate muscle bands of striped fibres, whose attachment on the ventral surface is confined to a circular spot, while on the dorsal surface it is lengthened out to a band at right angles to the length of the bug (see Plate XXIII, fig. 2).

These sets are very closely surrounded by the trachea trunks, and have many small branches ramifying through them. Contraction of these muscles would tend to help expiration. There do not appear to be any special inspiratory muscles.

Nervous System.

There is not much in the nervous system of the bug which calls for special mention, except that it is greatly condensed. The dorsal brain lies immediately behind the muscles of the pharynx, and hence just at the border between head and thorax. At the front and sides it is produced forward into the thick optic lobes. Below the brain and a little behind it, and thus in the pro-thorax, is found the sub-oesophageal ganglion, united with the other by the commissures, which, owing to the dorsal brain having projecting lobes (the antennal lobes), are very short. Through the ring thus formed goes the oesophagus, as well as the two main trunk tracheae which supply the head (Fig. 26). From the sub-oesophageal ganglion spring the nerves of the mouth parts, and from it also two nerves go backwards and link up with the thoracic ganglion situated in the fore part of the meta-thorax, which ganglion in the bed

bug is all that represents the theoretical chain of segmentally arranged nerve centres. It is rather a large mass, and is laterally bilobed, giving off nerves to the thoracic appendages. From its posterior end goes off the twin trunk which supplies the rest of the body. After the thoracic ganglion the nerve cord, which, although double, yet has the two cords so closely approximated as to appear single, has no visible ganglionic swellings. Along its length it is in close proximity to fat cells which almost obscure it in a well-fed bug, while in one which has had a long fast, the nerve cord is easily distinguished.

The structure of the eye has not yet been investigated by the writer, because repeated trials with different fixatives under varying conditions either rendered the chitin too brittle or else did not fix the contents of the eye. From a " paraffin embedding " point of view, the bed bug is very difficult to manipulate, as the chitin is so impervious to reagents. The writer after many failures only got results by slicing off a portion of the side of the abdomen after partial fixing so as to enable the reagents to penetrate. Without this the bug was never properly imbedded. Henning's solution, recommended in the Microtomist's Vade-Mecum as good for softening chitin, was not conspicuously successful with this insect.

The Fat Body.

Since the bed bug has often to pass long periods without any prospect of a meal, we would expect to find its fat cells well developed. They are in great profusion and for the most part are found in two rows of irregularly shaped cell masses, arranged down the sides of the abdomen. Their size varies greatly, according to the date of the last meal.

Besides the lateral major masses, there are collections of what are evidently fat cells round the heart and the nerve cord.

The Stink Glands.

Any one at all acquainted with bed bugs is aware that they can give rise to a most offensive smell. This is the secretion of a couple of glands (one on each side of the thorax), which open to the exterior on the metasternum. In the words of Messrs Douglas and Scott (*The British Hemiptera*, Part 1, page 2): " On each side (of the metasternum) anteriorly near the coxa, is the orifice of the internal sac containing the matter which gives to so many of the Hemiptera their disagreeable smell." These sacs vary very much in size. Occasionally they extend well down into the abdomen, and again they may be so minute as to be

almost invisible. Their condition cannot be foretold from the closest inspection of the living insect. The power of the smell bears a distinct relation to the size of the gland, being *very* strong when glands are well developed, and almost imperceptible when they are small. On one or two occasions the writer had mature specimens in which the glands could not be seen, and with them there was absolutely no smell, although all were dissected under the same conditions, and the writer's olfactory sense was as acute as usual. Not only do they vary in size but also in appearance, being smooth in surface when full, and very wrinkled when partially empty. The evil-smelling contents of these sacs are described by Landois as an " oily volatile fluid." What may be its nature the writer cannot say, but it does not seem to be an oil. When the sacs are ruptured under water there is no trace of an oily film on the surface, and the water gives the same sort of " jerk " as occurs when a drop of spirit is allowed to fall on its surface. As Landois says, when the dissection is made in alcohol the smell is far worse. When weak, the smell produced by the bug has been described as the smell of old dirty clothes. How the bug profits by the great strength of its well-known stench has yet to be discovered. If the authorities can be relied on, cockroaches eat them with gusto, and have seriously been suggested as a means of ridding premises of them, so that evidently the smell is no protection from enemies. Perhaps the principle, prevalent even to-day, that the more nauseous a medicine the more efficacious it is, was what induced Dioscorides to give nine bed bugs enclosed in a bean as a cure for fever ; while Pliny gave an infusion of the bodies of seven crushed bugs to arouse from impending coma.

The only good word ever spoken of the bug seems to be another remark of Pliny's that hens that have eaten one will not that day die from adder bite.

The odour is possibly of use to the bug when mating, and in the ordinary affairs of life it probably helps the social intercourse of the species in the gloomy recesses where they spend their lives, and in which, without a guide of some sort, they might have difficulty in finding each other. When kept in a close box they do not scatter themselves over its surface, but are always found gathered together in a solid mass, and this although the whole inside is equally dark and equally warm.

Sex and physical condition of the bug as regards nourishment, seem to have no effect on the size of the glands, as they have been found large and small in male and female, whether full-fed or famished. There would appear to be some evidence to show that cold reduces the

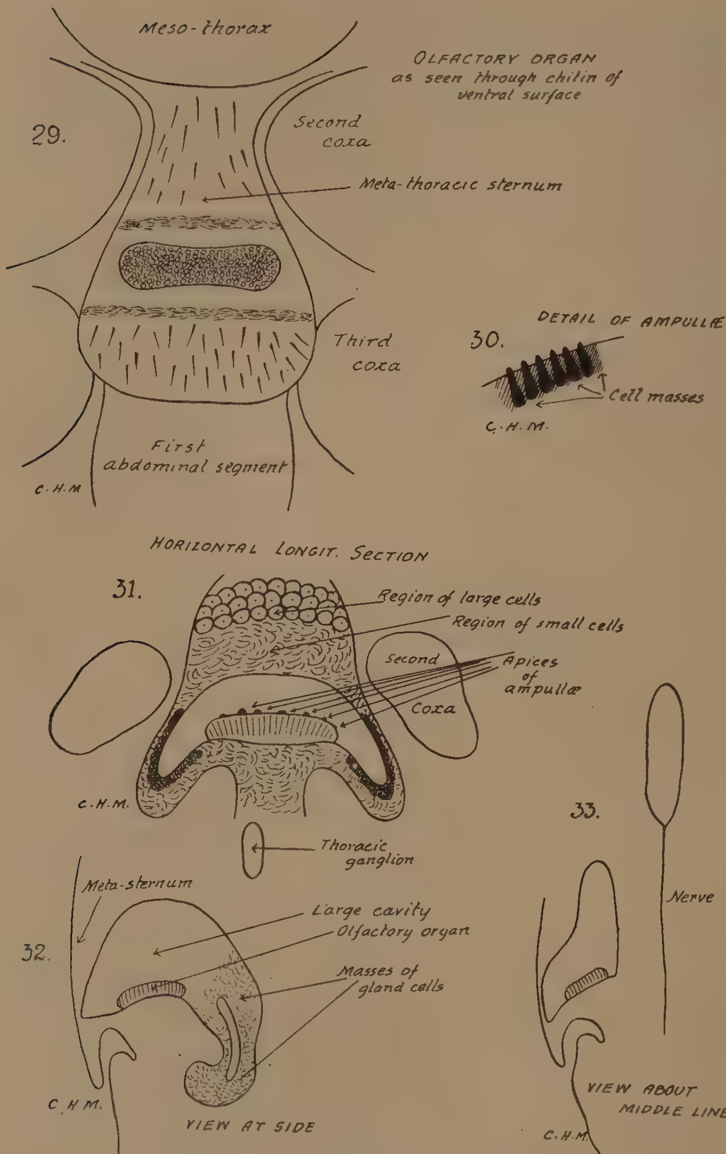
size of the glands, while heat increases it but hardly enough for one to dogmatise on the matter.

Landois' two remarks that the " preparation of the scent glands is difficult " and further on, " it is. . .extremely small, so no one is at all surprised that up to the present it has been entirely overlooked," are possibly due to the above noted fact about the size of the glands varying, a point which he does not mention.

The two glands have each, as already said, a separate opening to the exterior, besides which they have an intercommunicating passage (Figs. 13 and 31). This runs across on the ventral surface, and in the middle line under the meta-thorax opens out to a large chamber in which lies what, for reasons to be given later, would seem to be an olfactory organ.

It may be held as fairly certain that where individuals of any species have a characteristic odour, others of the tribe are able to recognise it. With many insects sensitiveness to this, especially in the case of the males at mating times, is so extreme as to be almost incredible, but the nature and even the place of the sensitive organ are often impossible to define.

Bugs would appear to have a sense of smell, for according to *A Manual of Entomology*, by Hermann Burmeister (trans. W. E. Shuckard, M.E.S.), " they are sensible to all kinds of odours : for example, citric acid, the sweat of horses, assafœtida, sulphur, etc., will drive them away for a time." The writer cannot vouch for the foregoing, but on examining the ventral surface of a bug which has been mounted whole in balsam, there is found at the level of the coxae of the middle pair of legs, or perhaps a little lower, a somewhat peculiar organ. Although inside the body, it can quite easily be seen through the chitin, if the creature has been for a considerable time without a meal, and hence has become more or less transparent when so mounted. The shape is shown by the sketch (Fig. 29). At first sight the most noticeable feature is its " dotted " appearance. This is due to the organ being composed of club-shaped—or as Landois says, flask-shaped—ampullae imbedded head downwards in its substance, the handles of the clubs, which project slightly above the surrounding surface, appearing like small buttons (Fig. 30). These ampullae, which are hollow, seem to be made of a very stiff material, possibly chitin, as in some microtome sections they have been lifted bodily from the surrounding tissue. Quite close to this organ, but rather anterior to it and more dorsal, is the thoracic ganglion which supplies it with nerves (Fig. 33). Comparing



Figs. 29—33. Sense organs of *A. lectularia.*

this organ in its general structure with the olfactory organs of the May Beetle, the Wasp, and *Caloptenus*, sketches of which (illustrating an article in Insect Life on *The Senses of Insects*, by the late Prof. C. V. Riley of America) are given (Fig. 34), it seems probable that it is this which gives the bed bug its power of distinguishing smells. The consensus of entomological opinion associates with the olfactory sense an organ of the above type of structure, *viz.* ampullae supplied with nerves.

As already said, the cavity in which the olfactory organ lies, communicates with the scent glands on either side (Figs. 13, 31). Moreover it

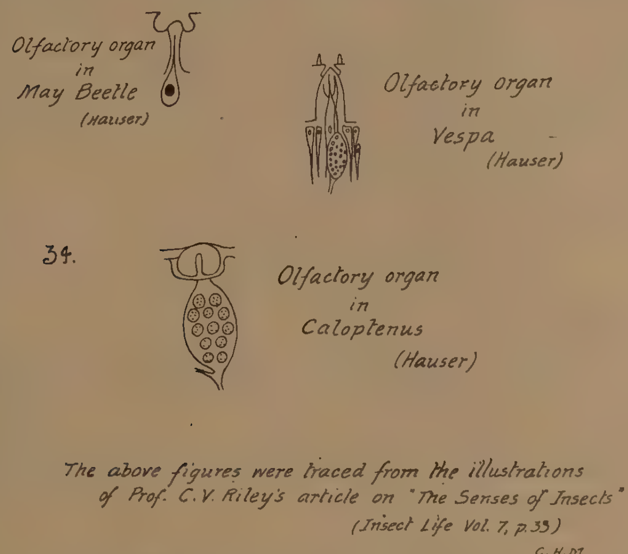


Fig. 34. Sense organs of other insects.

communicates with the exterior by an extremely small pore which lies in the middle line under the overhanging portion of the meta-thorax, and thus invisible from the ventral surface. How the sense of smell can be exercised by the bug is somewhat of a mystery, as the olfactory cavity not only communicates with the exterior but also with the glands, which secrete the same type of odour as the organ is evidently adapted to recognise. Possibly there may be a type of olfactory "tuning," to use a "wireless" term, which enables it to recognise outside odours. In no microtomed section made by the writer has he ever got any

appearance suggesting that the cavity is filled with fluid. His experiments with bugs have never yielded the oil drop from the central pore under the meta-thorax which Landois says can be seen.

There seems to be a peculiarity about the olfactory organ which the writer mentions with some reserve. When seen in section it is easily recognised, and is large enough to extend over a number of the sections, and since the ampullae stain deeply it is very noticeable, whether the section is transverse or longitudinal. When the bug is mounted whole in balsam it is also very distinct if seen at all. In his series of sections some show absolutely no trace of it, and this is not due to tearing of the section, for even if torn, the organ can easily be recognised although its position may be changed. A possible explanation which suggests itself is that the organ (which is common to both sexes) may become hypertrophied at times of sexual activity, and at other times be so small as to be practically invisible. As the scent of insects at such periods is much stronger than usual it may well be that in the bug a keener sense of smell may then also develop.

Landois asserts that the scent apparatus consists of:

(1) The gland,
(2) The bladders (for storage),
(3) The exit

(Plate XXII, fig. 2).

He describes as the scent gland the organ which, from its structure, the writer considers to be the olfactory organ; while what are in reality the scent glands Landois calls the storage bladders. The position he assigns to the "gland" (olfactory organ) as shown in his figure is quite wrong, as it is in the middle line, and, as already said, it is enclosed in the passage connecting the two glands ("reservoirs" according to Landois).

When discussing the exit he says: "Die Ausflussöffnung des Stinksackes befindet sich zwischen der Insertion des zweiten und dritten Beinpaares" (page 219, lines 12, 13)—while further on he says: "Die Oeffnung liegt jedoch versteckt und zwar unter jener Platte, welche ähnlich einem Processus xiphoideus vom Mesothorax her zwischen die Hinterbeine sich einscheibt" (page 221, lines 19–22).

It is thus difficult to understand what he actually means. In his figure he shows the opening of the glands as in the middle line (Plate XXII, fig. 2).

Figure 13 shows the scent and olfactory apparatus as seen by the writer.

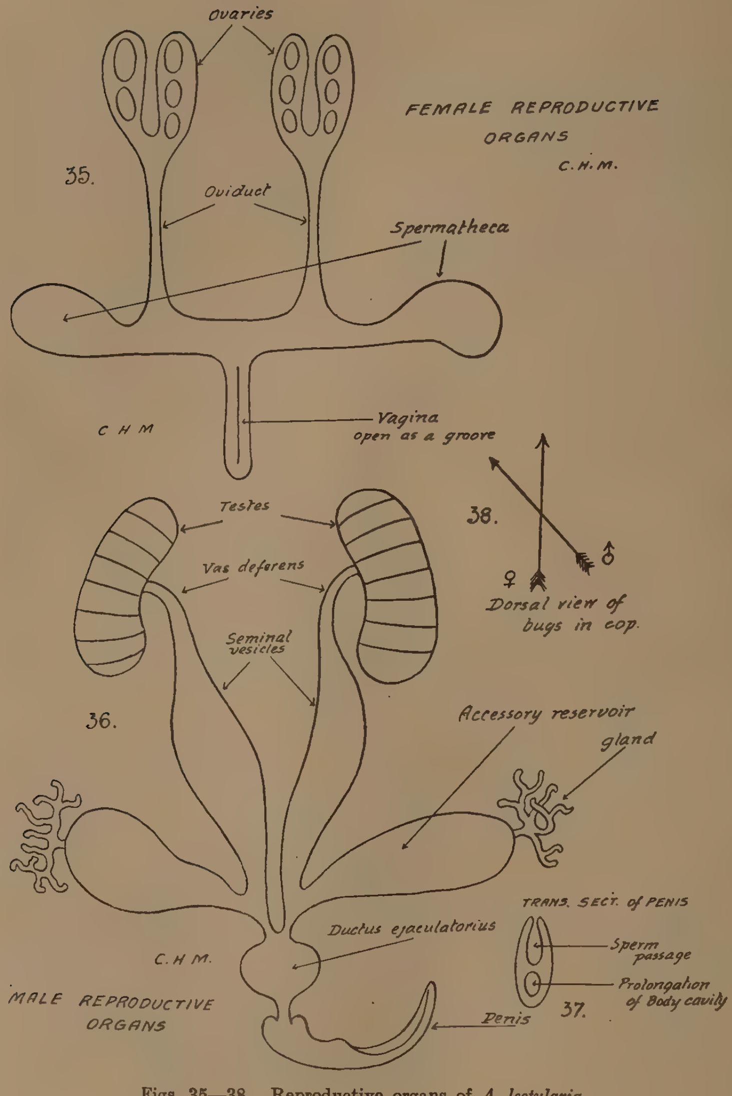
Reproductive System.

The reproductive organs of the male bug consist of testes, vasa deferentia, vesiculae seminales, accessory glands with their reservoirs, ductus ejaculatorius and the penis (Fig. 36). With the exception of the ejaculatory duct and penis all the foregoing are duplicate, so a description of one side suffices. The testes consist of seven large translucent white follicles in one plane united into a single mass, abundantly supplied with tracheae. In section these follicles give much the same appearance as would the section of a raspberry, were each drupe to have the same structure as the raspberry. All these open into the thin, short, uncoiled vas deferens which enlarges to form the vesicula seminalis. Usually this is quite tightly packed with sperms. At its base and to the outside is the accessory gland reservoir, which is a small pouch at whose distal end is the accessory gland itself. This consists of several branched filaments whose walls are composed of very glandular cells. Their function is supposed to be the supply of mucus which is mixed with the sperms as the latter are discharged. Next comes the ductus ejaculatorius, which is a median spherical-shaped portion into which the two halves of the reproductive glands open separately. From it rises a very short duct opening into the proximal end of the penis. This is a very strong hollow chitinous organ placed asymmetrically in the bug and pointing to the left. Usually it is carried concealed in a recess in the anal segment, but it is often seen slightly protruded.

The penis is curved in a horizontal plane, and ends distally in a blunt point, while at the proximal end it terminates in a manner which recalls the appearance of the shoulder articulation of the humerus. Normally it points forwards and to the left, the tip being the only portion usually visible. In its substance there are two parallel passages arranged (Fig. 37). Of these the inner one is open along its length and strongly suggests the poison fang of some snakes. Along this almost closed groove the sperms pass. The outer passage is a prolongation of the body cavity, such as we find in the maxillae and mandibles.

Since the penis is a lateral organ, the position during copulation is such as we would expect to find, namely that the long axis of the male bug is at an angle to that of the female. This is shown diagrammatically in Fig. 38.

The female bed bug would seem to lay comparatively few eggs.



Figs. 35—38. Reproductive organs of *A. lectularia*.

According to Southall, about fifty eggs are laid in each batch, and this would seem to be about correct, as the ovaries, which are seven egg tubes on each side, contain, at the most, three large ova in each of the fourteen. These do not show (as in the queen bee) all the stages from undifferentiated cells down to mature eggs, but all the ova are seen to be at the same stage of development. It may be inferred from this that the bug lays all her eggs at about the same time. According to Girault, a female bed bug, kept in captivity, will lay over 100 eggs in a period of approximately three months. This suggests that there are at least two laying periods per year, depending possibly on the presence of food and warmth, but as the writer has carried out no breeding experiments, he cannot vouch for Girault's remark.

Laying so few eggs at a time, the rapid multiplication of bed bugs is probably due to the fact that the eggs are laid, at each recurring period, in inaccessible places, and being thus undisturbed a large proportion come safely to the hatching. From the ovaries spring the oviducts. These are short tubes uniting ovary and vagina. At their bases on either side is a spermatheca for the storing of the sperms received from the male. At the level of the junction of the oviducts and spermathecae is the vagina, which is a narrow groove open for the major part of its length. It lies in the middle line between the two central plates, at whose lateral edges are to be found the ventral portions of segment seven. The bristles which arise from its edges are similar to the long thin one found at the base of the thick stumpy spine on the first tarsus of each foot. This groove receives the penis when the act of copulation takes place (Fig. 35).

APPENDIX.

The latest work on the bed bug appears in *A Textbook of Medical Entomology*, by Messrs Patton and Cragg, both of the King Institute of Preventive Medicine, Guindy, Madras, treating of various insects which are pests to man. One section deals with the bed bug, and the authors would seem to have laid undue weight on the authority of Landois, many of whose mistakes or defects of observation are repeated by them. The book was published in Madras in December, 1913, and in this country was not available until the end of February, after the foregoing paper was completed, and as it was thus too late for criticism in the paper itself, the writer would merely point out, in an appendix, what he considers to be some of the more outstanding inaccuracies.

It should be stated, however, that the bug treated by them is *rotundatus* and not *lectularius*, but as they say that there are "few divergences, except in the thorax, between this species and *lectularius*" (p. 499) he notes the discrepancies between his work and theirs, considering it unlikely that a mere difference in species would mean any great anatomical dissimilarity.

Messrs Patton and Cragg repeat Landois' errors when they describe the labrum as having two joints, and the labium four (p. 500), also when they say that the maxillae are unequal in length (p. 502).

According to them there is no epipharynx (p. 500).

The labium, is not, as they describe it, a hollow organ containing a continuation of the body cavity. It is quite solid apart from the tracheae in it.

The figure (Plate LXII, fig. 4) showing the cross-section of the stylet tube of *A. rotundata* is not as in *A. lectularia*, where the maxillae are corner pieces, as may be seen in the writer's sketch, and they do not anywhere in their length completely enclose the mandibles, as shown in the "Textbook" figure.

The statement that the legs "are not specially adapted to enable the bug to cling to its host" (p. 503) is obviously wrong when the spines, bristles, claws, ridged empodium, and coxal "notch" are considered (see Figs. 19–20).

There are three tarsal joints, and not four as the "Textbook" says. It would appear that the authors have mistaken the empodium for a tarsus joint, since they figure it (Plate LXII, fig. 6) but say that the bug possesses no such appendage.

When treating of the meta-thorax there is no reference to the two openings of the scent glands, which openings are easily seen, and are mentioned moreover by Messrs Douglas and Scott.

The first *four* abdominal segments have the previously mentioned ventral groove and the chitin thinner to allow of expansion after feeding. The "Textbook" says that only the first *two* are thus supplied.

The authors say that there are *five* pairs of plates making up the anal end of the ventral surface of the female, but this is certainly not so with *lectularia*, the number, counting the two portions of the seventh abdominal segment, being only *three* pairs and an odd one.

When discussing the sucking apparatus the "Textbook" omits any reference to the special shape of the crop, and merely says that it consists of "two plates superimposed upon one another in the transverse plane" (p. 514).

According to the "Textbook" the muscles moving the crop roof arise from the dorsal *and lateral* walls of the head. This last is wrong, as may be seen on reference to Figs. 25 and 26.

The authors omit all reference to the thickened "bottle neck" rim at the anterior end of the crop, through which the stylets pass, thus linking up their cavity with that of the pharynx, and they say "the means by which the food canal in the proboscis is connected with the pharynx has not been made out. They have no chitinous continuity" (p. 514).

The constrictions mentioned as being permanently on the stomach have been shown by the writer to be only temporary.

Their statement that the posterior end of the gut, *i.e.* the intestine, "never contains fresh blood" (p. 515) has been shown to be wrong, as also has been the suggestion that only in a fasting bug does the stomach contain air.

They make no mention of the bands of dark glandular cells on the stomach walls.

When discussing the malpighian tubules they say "their low insertion being a remarkable feature." What is meant the writer cannot say, as their position at the junction of intestine and rectum is quite normal.

As was the case with Landois, the authors failed to trace the ducts leading from the salivary glands to the reservoirs, and thus they say that there are "two pairs of salivary glands."

Messrs Patton and Cragg describe as follows a complicated salivary pump, the essentials of which are:

"A vase-like cup of chitin with its narrow end directed to the point at which the two mandibles come in contact with one another. The broad posterior end is closed in by a membrane in the middle of which is inserted a short rod of chitin."

"Contraction (of muscle fibres) pulls the rod and therefore the membrane away...creating a negative pressure, which negative pressure will therefore result in drawing the saliva from the glands into the pump."

"Presumably there is some valve which prevents ingress of blood into the pump when in action."

"There are no muscles which could force the piston rod forwards" (pp. 517–518).

There are on Plate LXIV two sketches relating to the above (figs. 4 and 6). Fig. 4 is the pump of *A. rotundata*, while fig. 6 is that

of *Conorhinus* "seen from the dorsal aspect in a cleared specimen" (the quotation is taken from letterpress describing figure)—that is, a bug mounted whole, and hence the figure is of the view when seen *through* the chitin of the head.

The writer has dissected scores of bugs and never once has he come across the salivary pump as described by Messrs Patton and Cragg. He has mounted bugs whole in balsam after having cleared them, and he has got a preparation similar to that of *Conorhinus* as figured in the book, and would give the following explanation of the "salivary pump" as seen by the authors. "The vase-shaped cup of chitin" with its narrow end to the anterior is the pharynx pump or crop. The "chitin rod" ("piston rod" as they call it on p. 518) is the oesophagus.

The writer's preparations always show the "chitin rod" accompanied by a trachea (not figured by Patton and Cragg) on either side. Microtome sections and dissections have never yet shown the presence of a "chitin rod." On boiling the head of *Acanthia* in potash the only structures left after the operation, are the lancets and the pharynx pump, there being absolutely no trace of any "vase-like cup of chitin" nor of any "piston rod" of like material.

As usually seen, the effect is as if the "chitin rod" had been pulled backwards, and in this condition the tracheae are straight. When the "rod" has been brought forwards, the tracheae are bent towards the middle line.

Why the oesophagus should be thus drawn forwards the writer cannot say, but so seldom is it seen in mounted specimens in this position, that it would appear to be the exception rather than the rule, at least after death (see Figs. 39 and 40).

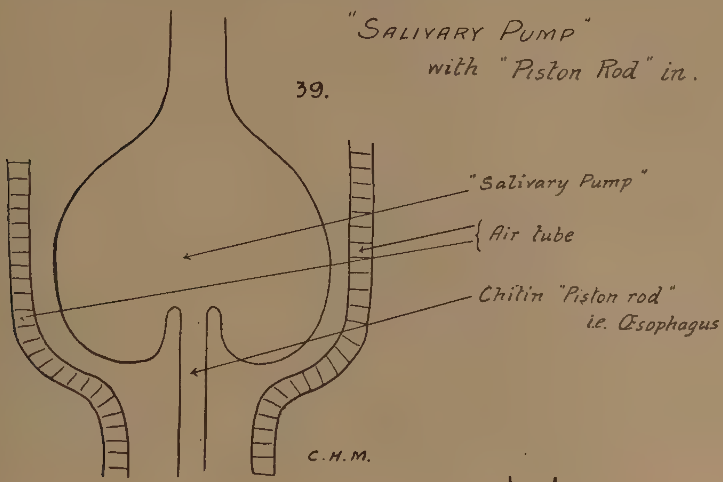
Male. The testes in *A. lectularia* are not pyramidal as is said to be the case in *A. rotundata*, but are arranged in seven masses more or less rectangular, placed side by side, forming a rather reniform-shaped structure.

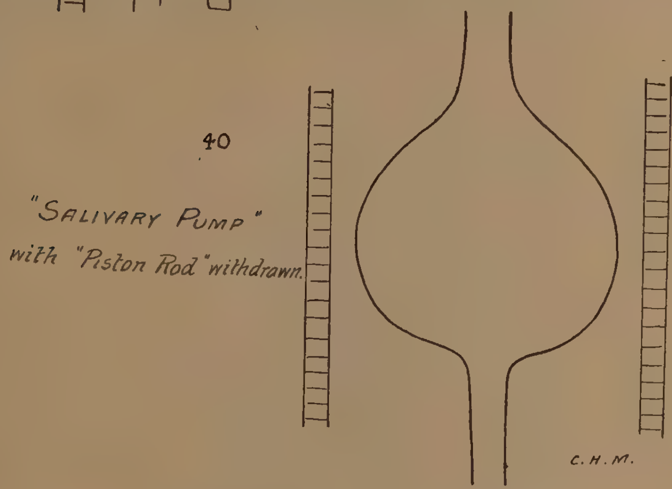
There is no sign of the accessory lobe of the testis, although Messrs Patton and Cragg say it is "of constant occurrence in *A. rotundatus*."

Possibly the fact that their figure (Plate LXV, fig. 1) shows the part ordinarily known as the "ductus ejaculatorius" labelled "seminal vesicle" is due to a printer's error. In *A. lectularia* the "seminal vesicle" is found in the lower portion of the "vas deferens" before it receives the "accessory" gland.

Female. In *A. lectularia* all the ova appear to ripen at about the same time, the writer never having seen ova at various stages of

development, as is said to be the case with *A. rotundatus*. *A. lectularius* possesses two spermathecae, whereas Patton and Cragg say "there are certainly none in *rotundatus* though...there are accumulations of





Figs. 39, 40. Salivary glands of *A. lectularia.*

sperms in the position in which one would expect to find spermathecae, which might easily be mistaken for them" (p. 519).

With reference to the "Organ of Berlese" the writer would like to point out that it would be a matter of great difficulty, if not utter impossibility, for the male bug to insert his penis in the external opening to this organ, as the authors say is done. The opening is found on the *right* side of the ventral region of the abdomen of the female. The penis lies in a shallow pocket on the left, so that it points to the *left* and forwards.

When copulating the position is that the male mounts the back of the female and puts his long axis to that of the female in the relative position that a line drawn on a clock face from 10 to 4 (male) bears to one drawn from 12 to 6 (female) (Fig. 38). The writer fails to see how an organ so placed and surrounded by hard chitin as is the penis, can reach over to the right side of the bug and forwards to the opening of the "Organ of Berlese," as Messrs Patton and Cragg affirm it does, for they say: "From the position of the male it is impossible for his penis to reach the genital opening of the female, whereas he is placed precisely as one would expect if his intention were to introduce it into the opening of Berlese's organ, for he is invariably on the right side of the female and just sufficiently far forwards to bring his posterior segments on a level with the opening." "The operation is carried out so quickly that it is not possible to obtain final proof by killing the pair *in cop.*, but repeated observations on many separate occasions have left no doubt in the minds of the writers that the male organ is actually introduced into the opening of Berlese's organ" (pp. 521–522).

The position of the male *rotundatus* in copulation would seem, as described by Messrs Patton and Cragg, to be much further forward than with *lectularius*, but in spite of this the writer hesitates to accept the idea that, with insects so closely allied, a different organ would be brought into play in the process.

The description of the scent glands in the "Textbook" is practically that of Landois, so the same criticisms hold. In their description on p. 523 they say that the exit is under the metasternum, while on p. 481 they speak of "the ducts opening by means of an orifice on each side of the metasternum."

They do not touch on the Respiratory, Nervous, or Circulatory systems.

Fig. 1.

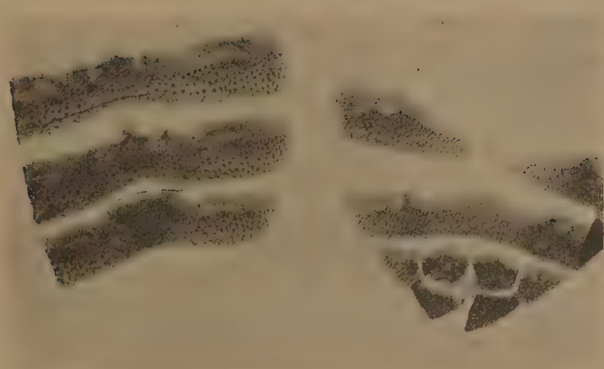


Fig. 2.

PLATE XXII.

The letterpress accompanying Landois' plates.

PHOTOGRAPH 1 OF LANDOIS' PLATE XI.

Fig. 1. Kopf der Wanze von unten mit extirpirter Unterlippe: *l*, die zweigliedrige Oberlippe; *r*, der Stechapparat; *mm*, die Basaltheile der Mandibeln; *mx*, die Basaltheile der Maxillen; *K*, Der Kropf; *a*, Antenne; *o*, Auge.

Fig. 2. Die Mundwerkzeuge der Wanze gegen den Thorax eingeschlagen: *lb*, Unterlippe mit dem Stechapparate; *p*, Prothorax; *ii*, erstes Beinpaar; *l*, Oberlippe.

Fig. 3. Die Mundwerkzeuge der Wanze von unten, die Unterlippe nach vorn hin horizontal ausgestreckt: *l*, Oberlippe; *lb*, Unterlippe.

Fig. 4. Endtheil des Stechapparates: *md*, eine Mandibel von der Seite gesehen; *mx*, *mx*, die beiden Maxillen.

Fig. 5. Die Spitzen der beiden Mandibeln von oben gesehen.

Fig. 6. Der Nahrungscanal: *O*, Oesophagus; *M*, Magen; *Dd*, Dünndarm; *Dk*, Dickdarm; *MG*, Malpighi'sche Gefässe; *Sp*, Die kleinen kugelförmigen Speicheldrüsen.

Fig. 7. Secretionszellen des Magens.

Fig. 8. Ein Stückchen des Malpighi'schen Gefässes mit den Secretionszellen.

Fig. 9. Anfangstheil des Stechapparates und der Kropf: *St*, Stechapparat sich zusammensetzend aus; *md md*, den beiden Mandibeln und; *mx mx*, den beiden Maxillen; *k*, Kropf; *o*, Einmündung der Speicheldrüsen.

Fig. 10. Die grosse gelbgrüne kugelförmige Speicheldrüse mit den Secretionszellen: *a*, der gespaltene Ausführungsgang; *b*, das Muskelfasern enthaltende Aufhängeband.

PHOTOGRAPH 2 OF LANDOIS' PLATE XII.

Fig. 11. Die kleine kugelförmige Speicheldrüse: *b*, Das Band, welches die Drüse und den Magen heftet; *a*, Der Ausführungsgang.

Fig. 12. Die schlauchförmige Speicheldrüse: *m*, die sie umgebenden Muskelfasern.

Fig. 13. Die verästelte Speicheldrüse.

Fig. 14. Der Stinkapparat: *Sd*, die Stinkdrüse; *Ss*, der Stinksack; *A*, der Ausführungsgang.

PLATE XXIII.

Fig. 1. Photograph of the dorsal surface of three adjoining segments of the exoskeleton of the abdomen of the Bug showing the bristles and bristle pits.

Fig. 2. Photograph of dorsal (left) and ventral (right) surfaces of the abdominal exoskeleton of the Bug to show the shape of the muscle attachments.

THE MORPHOLOGY AND BIOLOGY OF *HERPETOMONAS PATELLAE*, N. SP., PARASITIC IN THE LIMPET, *PATELLA VULGATA*, TOGETHER WITH REMARKS ON THE PATHOGENIC SIGNIFICANCE OF CERTAIN FLAGELLATES FOUND IN INVERTEBRATES.

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Beit Memorial Research Fellow.

(*From the Quick Laboratory, Cambridge.*)

(With 17 Text-figures.)

CONTENTS

Introduction.

Many herpetomonads have been recorded from various insects belonging chiefly to the Diptera and Hemiptera, but until the present instance, members of this genus of flagellates have not been found in the Mollusca and very few in other Invertebrata. Also the habitat of the herpetomonads has usually been either the main alimentary tract or the haemocoel of their hosts. Digestive glands hitherto have not been recorded as being infected.

Recently, during examinations of a number of the edible limpet, *Patella vulgata*, I was fortunate in finding one infected with a small number of minute herpetomonads, the alimentary tract and digestive

gland (or "liver") of the mollusc being infected. Other limpets were examined for herpetomonads, about fifty in all, and eight per cent. of the total examined were found to be slightly infected. The range of the genus Herpetomonas is thus extended to the Mollusca, and the digestive gland is added to the organs of a host that may harbour such parasites. The name *Herpetomonas patellae* is proposed for this new flagellate.

I have much pleasure in thanking Professor Herdman and Mr H. C. Chadwick for their kindness in facilitating my researches at Port Erin, Isle of Man, in Nov. 1913, when the parasite was found. Mr Chadwick greatly aided me in obtaining material. Observations were continued near Brighton in May 1914. Preparations were further studied and the research was completed in Cambridge in the laboratory of Professor Nuttall, to whom my thanks are due.

Observations on living HERPETOMONAS PATELLAE.

The flagellate herpetomonads appear as very small, somewhat refractile organisms and move jerkily, the body being sharply flexed and then straightened and the flagellum lashing vigorously. Swirling movements, suggestive of those of a boomerang, are common. The flagellum is thick and is easily seen in life. It is often as long as the body. The flagellar end is forwardly directed in movement. The parasites showed no tendency to collect in groups and neither division rosettes nor aggregations were observed. A few dividing forms were watched and multiplication by simple longitudinal fission was the only mode of increase seen. A few young flagellates were found in the stomach of the limpet; full grown forms were most abundant in the "liver." A few were observed in the rectum and faeces.

The part of the "liver" adjacent to the stomach also contained ovoid, non-flagellate forms of the parasite. Some of these in a fresh preparation were watched for several hours, and the gradual production of a flagellum with subsequent elongation of the body was seen in individual cases. The flagellum was often formed before the distal or posterior end of the body lengthened. A few thicker walled post-flagellate forms were found in the distal end of the "liver," as well as in the rectum.

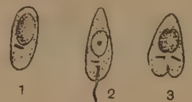
Morphology.

The morphology was studied both in the living organism and in stained preparations. Smears of the gut contents and of the digestive

gland of the limpet were fixed wet in Bouin's fluid, in osmic acid vapour followed by absolute alcohol, or in formalin vapour. They were stained by Delafield's haematoxylin, by glycerine haematein or by Giemsa's solution. Fixation by Bouin's fluid, followed by haematoxylin staining was best.

The parasite exhibits the usual three phases common among flagellates in its life-cycle.

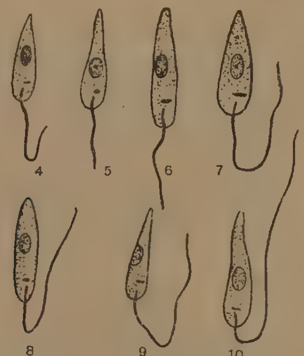
A. Pre-flagellate forms. The pre-flagellates (Fig. 1) are ovoid bodies measuring from 2 μ to 3 μ long, and are about 1 μ to 1·5 μ broad. The nucleus is oval, showing sometimes a central karyosome (Fig. 2), but more often having the chromatin evenly distributed (Fig. 1).



Figs. 1–3. *Herpetomonas patellae.* Pre-flagellate, developing young flagellate and dividing pre-flagellate forms. × 3000.

The blepharoplast is barlike and homogeneous. Some specimens showed a chromatophile area from which the flagellum develops. The growing pre-flagellate shows an elongation of the end near the blepharoplast. A chromatoid thread is gradually differentiated, which pushes the periplast forward and then projects as a free flagellum (Fig. 2).

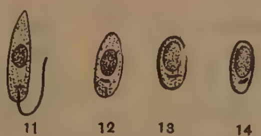
B. Flagellate forms. The flagellates (Figs. 4–10) measure from 5 μ to 7 μ in body length and are about 1 μ broad. The free flagellum is sometimes somewhat longer than the lanceolate body (Figs. 7–10).



Figs. 4–10. *Herpetomonas patellae.* Various aspects of the flagellate stage. × 3000.

The flagellar end is rather blunt (Fig. 6). The posterior or aflagellar end is more pointed in some specimens (Figs. 7, 9), but is not markedly so in others (Figs. 6, 8). The nucleus exhibits the same two types as are seen in the pre-flagellates, namely, those with the chromatin distributed in granules (Figs. 6, 10) and those with it concentrated as a karyosome (Figs. 5, 7). In forms about to divide, the nucleus is always granular in character and the karyosome is not seen.

C. Post-flagellate forms (Figs. 11–14). These resemble the pre-flagellates and are about the same size, but have somewhat thicker



Figs. 11–14. *Herpetomonas patellæ.* Development of the post-flagellate form. ×3000.

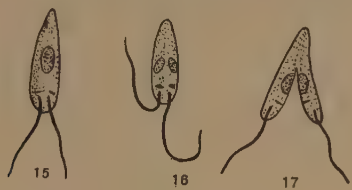
cyst walls. The nucleus most frequently shows homogeneous chromatin (Figs. 11–14), but occasionally a karyosome is present. The blepharoplast usually is near the nucleus (Figs. 12–14); in a few specimens it was superimposed.

The post-flagellate form of *H. patellae* is derived from the flagellate by the retrogression and absorption of the flagellum (Fig. 11), accompanied by the gradual concentration of the body cytoplasm around the nucleus. The wall at first is gelatinous and swells slightly if warm distilled water be added to the preparation. No swelling occurs in preparations made with physiological salt solution at room temperature. The gelatinous cyst wall gradually contracts and forms a varnish-like sheath in the final resting condition (Figs. 12–14).

Multiplication.

Multiplication occurs by binary fission of either the pre-flagellate (Fig. 3) or flagellate (Figs. 15–17) parasites. The division of the pre-flagellate (Fig. 3) is initiated by that of the blepharoplast. The nucleus gradually constricts into two. The cytoplasm segregates to either side of the body so that a continuous vacuole appears from end to end of the pre-flagellate. A cleft is next seen near the blepharoplast and gradually extends backwards to the posterior end. Slight movements of the body occur. No division rosettes of the pre-flagellates of *H. patellae* were found.

The division of the flagellate forms of *H. patellae* (Figs. 15–17) follows on the same lines as that of *H. jaculum*, *H. lygaei*, *H. pediculi* and other herpetomonads. The order of division is blepharoplast, flagellum,



Figs. 15–17. *Herpetomonas patellæ.* Dividing forms. × 3000.

nucleus, cytoplasm. Division of the flagellum, which may be unequal, was observed in the living organism. No budding phenomena, such as those described by some authors for the flagella of dividing *H. muscae domesticae*, have been observed. No division rosette formation has been seen in *H. patellae*, nor were aggregation rosettes found. Perhaps this was due to the scanty infection encountered.

Nutrition of H. PATELLAE.

Apparently the larger number of the herpetomonads were nourished on the liver fluid surrounding them, which they absorb by osmosis. There was no evidence of the existence of a cytostome, cytopharynx or cytopyge in *H. patellae*. A recent suggestion that the myoneme striations of some herpetomonads were really the edges of a cytopharynx is inaccurate so far as *H. patellae* and *H. jaculum* are concerned. Though continuous observations were made, no organised substances such as bacteria or food particles, either in process of ingestion or digestion, were seen in the body of *H. patellae*. Nutrition seems to be by passive absorption of fluid food by the general body surface, and resembles that of other insect flagellates and such organisms as trypanosomes.

Mode of Transmission.

A few post-flagellate forms were obtained from the anal portion of the gut of the limpet. Flagellate forms were not common in the gut, though a few flagellates were seen in the rectum and freshly shed faeces. The occurrence of post-flagellate forms in faecal matter suggests that the mode of infection is contaminative. The paucity of infected material prevented further experimental work being undertaken. The flagellates

doubtless gain access to the digestive gland ("liver") by passing along the numerous small ducts that finally unite into one leading from the "liver" to the stomach of the limpet.

Systematic Position.

The flagellate parasitic in *Patella vulgata* is a member of the genus Herpetomonas as originally defined. It is considered to be a new species, *H. patellae*. It shows the following characteristics among others. *H. patellae* is much smaller than any other Herpetomonas yet described, its body being only 5 μ to 7 μ long. The relatively large size of the non-flagellate stages of the organism is not common among other herpetomonads. The movements of *H. patellae* are more jerky than those of most other herpetomonads and the "boomerang" movement is unlike that described for any other species, so far as I am aware. Its occurrence in the digestive gland of a mollusc is believed to be unique at present.

Remarks on the pathogenic significance of certain Flagellates found in Invertebrates.

During the last few months, thanks to the interesting investigations of Laveran and Franchini, the importance of herpetomonads and Crithidia has greatly increased from the pathogenic point of view. Laveran and Franchini's first series of experiments (Sept. 1913) showed that *Herpetomonas ctenocephali* (Fantham), parasitic in the gut of the dog flea, *Ctenocephalus canis*, could be inoculated intra-peritoneally and otherwise into white mice with fatal results. Leishmaniform parasites were found in the blood and internal organs of the mice, especially in the liver. A very few flagellate parasites only were seen in the inoculated vertebrate. Rats and a dog were also inoculated. The dog died, leishmaniform parasites being found in the internal organs. The infection could be maintained by successive inoculations into clean vertebrates.

Similar experiments were made with *Crithidia fasciculata* (Léger), parasitic in the gut of *Anopheles maculipennis*. The experiments began with the successful inoculation of the contents of the digestive tract of parasitised Anopheles into rats and mice.

Further experiments were undertaken with *Herpetomonas pattoni* (Swingle), parasitic in the gut of the rat flea, *Ceratophyllus fasciatus*. White rats and white mice became infected when kept in contact with numerous parasitised rat fleas. It was then shown that the mode of

infection of the rodents was by eating the parasitised rat fleas, the incubation period being two to three weeks—that is, longer than by intra-peritoneal inoculation.

Mice were also infected by way of the digestive tract with *Crithidia melophagia* (Flu), a parasitic flagellate of the sheep ked, *Melophagus ovinus*.

The authors' last series of experiments (July 1914) showed that mice can be infected by ingesting the faeces of rat fleas containing *H. pattoni*; also that inoculation of *Crithidia fasciculata* into a white mouse produced cutaneous lesions resembling those of Oriental Sore. As was stated by Fantham and Porter (Dec. 1913), when commenting on the earlier work of Laveran and Franchini, these experiments signify "leishmaniasis in the making." The spleen was found to be enlarged in the various experimentally infected animals. Flagellate parasites were very rare indeed in the experimental animals and were even absent in some cases, but leishmaniform and uninucleate rounded elements occurred in the red blood corpuscles and free leishmaniforms were observed. Further, it is possible that the canine kala-azar occurring in the Mediterranean region is really a canine herpetomoniasis due to *Herpetomonas ctenocephali*.

These experimental results should be compared with certain established facts, of which two may be specially mentioned: (1) The various species of *Leishmania* in culture grow into herpetomonad flagellates. (2) Franchini (1913) described a herpetomonad parasite obtained post mortem from a human subject who had long lived in Brazil. He named the parasite *Haemocystozoon brasiliense*, though as Brumpt (1913) says, it might have been placed, probably, in the genus *Herpetomonas*. A third point, which is an inference from Laveran and Franchini's experiments, and is mentioned by the authors themselves, is that *Leishmania* and the trypanosomes probably arose from the flagellates of invertebrates. Fourthly, it should be remembered that leishmaniform elements have been described by Moore and Breinl (1907) and by Fantham (1911) as part of the life cycle of trypanosomes in vertebrate hosts. These leishmaniform elements or "latent bodies" occur in the internal organs. Their occurrence serves further to point out the close genetic and even phylogenetic relationship between *Herpetomonas*, *Leishmania*, *Crithidia* and *Trypanosoma*.

As *Herpetomonas patellae* occurs in an edible invertebrate, it may be remarked that such flagellates are possibly not without pathogenic properties, if they should find their way alive in sufficient numbers

into a vertebrate. Without wishing to be an alarmist, it is well to remember the pathogenic importance of herpetomonads and Crithidia, originally occurring as natural parasites of invertebrates, but possessing the power of adaptation to life in the sanguineous organs of vertebrates.

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